CHEMICAL CHARACTERIZATION OF OLIVE OILS FROM KARABURUN PENINSULA

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ABSTRACT

CHEMICAL CHARACTERIZATION OF OLIVE OILS FROM KARABURUN PENINSULA

Chemical characteristics of olive oils produced from Erkence olive variety that is mainly grown around Karaburun Peninsula of İzmir have not been investigated thoroughly although this variety has high oil content and ripens earlier compared to other olive types. Identifying the chemical characteristics of olive oils could be useful to obtain geographical indication labelling for olive oils produced from this variety.

Aim of this study is to determine some important chemical characteristics of olive oils from Erkence olive variety produced in Karaburun region and to investigate the differences in olive oils that come from various parts of the Peninsula using chemometric techniques as principal component analysis (PCA) and partial least square (PLS) regression. For this purpose, total phenolic content, fatty acid profile, phenolic profile, total carotene and chlorophyll contents and oxidative stability of 64 olive oils were determined. FTIR spectra for these oils were also evaluated. According to PCA results, classification with respect to geographical origin was relatively more successful with FTIR analysis while phenolic and fatty acid profiles did not result very satisfactory separation between regions.

Moreover, FTIR spectra and various chemical parameters were used to predict oxidative stability of all olive oil samples. Oxidative stability was predicted successfully from IR spectra whereas prediction from chemical parameters was not that successful. IR spectra were also used to predict various chemical parameters. As a result of PLS regression; chlorophyll and carotenoid, some individual phenolic components (p-coumaric, hydroxtyrosol) and some major fatty acids (oleic, linoleic and palmitic) were predicted.

ÖZET

KARABURUN YARIMADASI ZEYTİNYAĞLARININ KİMYASAL KARAKTERİZASYONU

İzmir'in Karaburun Yarımadası ve çevresinde yoğun olarak yetişen Erkence zeytin çeşidi diğer zeytin çeşitlerine gore daha fazla yağ içeriğine sahip olmasına ve daha erken olgunlaşmasına rağmen bu zeytinden elde edilen yağların kimyasal özellikleri derinlenmesine incelenmemiştir. Coğrafi belirteç etiketi alınabilmesinde bu zeytinyağlarının kimyasal karakterlerinin tanımlanması yardımcı olabilir.

Bu çalışmanın amacı asal bileşenler analizi ve kısmi en küçük kareler regresyonu gibi kemometrik yöntemler kullanılarak Karaburun yöresindeki Erkence çeşidi zeytinden elde edilen yağların bazı önemli kimyasal özelliklerini saptamak ve Yarımadanın çeşitli yörelerinden gelen zeytinyağlarının farkını incelemektir. Bu amaç için 64 adet zeytinyağı örneğinin toplam fenol içeriği, yağ asiti profili, fenolik profili, toplam karoten ve klorofil içeriği, oksidatif kararlılığı belirlenmiştir. Fourier dönüşümlü kızılötesi spektrası da değerlendirilmiştir. Asal bileşenler analizi sonuçlarına göre coğrafi kökene dayalı sınıflandırma göreceli olarak en başarılı şekilde Fourier dönüşümlü kızılötesi spektra analiziyle belirlenmiş iken fenol ve yağ asiti profilleri bölgeler arası ayrımda çok başarılı sonuçlar vermemiştir.

Ayrıca, çalışılan yağların tümünün oksidatif kararlılığı Fourier dönüşümlü kızılötesi spektra ve çeşitli kimyasal parametreler kullanılarak tahmin edilmiştir. Oksidatif kararlılık kızılötesi spektradan başarılı bir şekilde tahmin edilirken çeşitli kimyasal parameterlerden tahmin o kadar da başarılı değildir. Kızılötesi spektra başka kimyasal parametreleri tahmin etmede de kullanılmıştır. Kısmi en küçük kareler regresyonu sonucunda klorofil ve karoten, önemli yağ asitleri (oleik, linoleik ve palmitik) ve bazı fenolik maddeler (p-coumaric, hydroxtyrosol) tahmin edilmiştir.

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CHAPTER 1

INTRODUCTION

Olive oil, extracted from the fruit of olive tree, is known for its precious nutritional, functional and sensorial qualities (Matos et al., 2007). Olive oil consumption has been increasing in recent years due to its balanced unsaturated fatty acid content and presence of other functional groups such as phenolic compounds, tocopherols and chlorophyll (Temime et al., 2008).

Extra virgin olive oil is defined as the olive oil which is produced only by mechanical processes like crushing, malaxation and centrifugation without any further chemical treatment. Since no refinement process is involved in its production, organoleptic and nutritional value of olive oils is well preserved. Also its defense mechanism against oxidative stress is protected (Angerosa et al., 2000; Mateos et al., 2005; Sánchez-Perona et al., 2006).

Olive oil is composed of various chemical components; as major saponifiable components glycerol (approximately 97-98%); free fatty acids, triacylglycerol, and phospholipids, and around 2% minor unsaponifiable part consisting of squalene, tocopherols, volatiles, pigments (chlorophylls and carotenoids) and phenolic compounds (Boskou, 1996). Each olive variety has its own characteristic chemical composition and naturally the chemical structure of olive oil obtained from those are highly variety-specific. Chemical parameters of olive oil are affected by the environmental factors such as soil and climate, cultivation stage mainly maturity degree and harvesting time, agronomic conditions; irrigation regime and fertilization, technological factors like extraction system and post-harvest storage conditions. All of these conditions make it difficult to characterize olive oils with low number of chemical compounds; therefore, samples must be identified with large number of variables (Aparicio & Luna, 2002). Evaluating huge data cluster is impossible with univariate statistical methods; for this reason, multivariate methods need to be applied to numerous variables to obtain meaningful interpretation. There are various approaches in multivariate analysis and one of these, principal component analysis (PCA),could indicate the correlation between observations (samples) and geographical origin or

variety of olive oil. Partial least square (PLS) analysis is used mainly for quantification purposes as well as for classification. Classification of olive oils with respect to variety and geographical location is really important due to the fact that quality and uniqueness of olive oils are attributed to specific region where olives grow; therefore, in order to guarantee the quality of olive oil, two different certification systems were created by European Union (EU) and are known as Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). PDO assures; foodstuff produced, processed and prepared within the specified geographical region while PGI stands for at least one of the mentioned steps are occurred in the specified geographical region. These geographical labelling systems attribute the quality of the product to its geographical region, contribute to traceability, help prevention of adulteration, add extra value to the product, and protect producer and consumer at the same time (Babcock et al., 2004). In France, a similar approach of geographical labelling exists as Registered Designation of Origin (AOC in French); main idea is similar to PDO but no chemical parameter is set except for acidity and it is based on sensorial evaluation whereas it is not enough alone for geographical classification (Ollivier et al., 2006). In Turkey, Turkish Patent Institute is the responsible organization for geographical labelling. PDO defined as "Menşe İşareti" and PGI defined as "Mahreç İşareti" in Turkish (Şahin, 2013). In this system, there are also some criteria like physical, chemical and microbiological specifications but not as efficient as European labelling system.

In the literature there are many studies using different chromatographic; (Alkan et al., 2012), and IR spectroscopic (Bendini et al., 2007) methods that focus on different chemical compounds of olive oil to provide differentiation with respect to cultivar or geographical origin in combination with chemometric methods.

The aim of this study is to investigate the differentiation of olive oils from Karaburun Peninsula according to geographical region divided into 9 areas using different data sets. One data set was established by Fourier transform infrared spectroscopy (FTIR) while fatty acid profile was determined by gas chromatography (GC) analysis and individual phenolic compounds were detected by high pressure liquid chromatography (HPLC) system. Quantitative parameters like total phenol content (TPC) and chlorophyll and carotenoid contents were determined spectrophotometrically while oxidative stability (OS) was obtained by Rancimat apparatus. Data were analyzed using Principal Component Analysis (PCA) and Soft Independent Modeling of Class Analogy (SIMCA) to see how well the olive oil samples are differentiated with respect

to their chemical characteristics. Apart from the classification purposes FTIR profile was also used for prediction of some chemical parameters (chlorophyll and carotenoids, OS, TPC, phenolic content and fatty acid profile). Furthermore, oxidative stability was also predicted from combination of various chemical parameters in combination with PLS.

CHAPTER 2

LITERATURE REVIEW

2.1. Olive Oil Brief History and Marketing Information

The olive tree (*Olea europea* L.) is well-adapted to Mediterranean countries and was firstly cultivated approximately 6000 years ago in the east Mediterranean area (Luchetti, 2002).

Olive oil production was started with the invention of screw press by Greeksand this system was improved by Romans. Until the middle ages, there was a decrease in olive oil production due to the fall of Roman Empire. In the middle of 1900's, importance of human power in olive oil production started to decrease, and new mechanical extraction systems appeared (Harword & Aparicio, 2000).

Unique chemistry of olive oil is associated with its positive contribution to human health; as a consequence, longer life of people whose basic diet is Mediterranean type is attributed to foods in this diet. In this trend, production and consumption of olive oil without any chemical treatment is becoming more popular (Morello et al., 2003).

Marketing of olive oil is a huge sector and major actors in this sector are Mediterranean countries which produce most of the olive oil in the world. Spain, Italy and Greece are the main producers in Europe as well as in the world. According to International Olive Oil Council (IOOC, 2014) Spain is the greatest olive oil producer in the world with the value of 1,216,100 t followed by Italy and Greece (455,800 t and 317,600 t, respectively) (Table 2.1). In terms of percentages, Spain possesses by itself nearly half of the production in the world. Turkey is in the sixth place in terms of production. While in olive oil consumption, France and U.S.A are revealed as important importers with the consumption value of 108,700 t and 271,300 t, orderly whereas their production rates are really low (<0.2%). In addition, Italians are consuming more than their production while the rest of the countries are consuming less compared to their production. The latest values from IOCC show that Turkey produces 180,000 t olive oil in 2013/2014 crop season while its consumption is 150,000 t.

Table 2.1. Average consumption and production of olive oil worldwide between 2007 2013 seasons in weight (x10^3 tonnes) and in percentages (Source:International Olive Oil Council (IOOC) 2014)

Countries	Production		Consumption	
	weight (t)	%	weight (t)	%
Spain	1216.10	42.48	543.40	18.45
Italy	455.80	15.92	658.50	22.35
Greece	317.60	11.09	224.80	7.63
Tunisia	167.00	5.83	34.30	1.16
Syria	159.30	5.56	118.70	4.03
Turkey	149.20	5.21	124.00	4.21
Morocco	110.00	3.84	96.00	3.26
France	5.30	0.19	108.70	3.69
U.S.A	4.30	0.15	271.30	9.21
Others	278.20	9.72	766.20	26.01

2.2. Production of Olive Oil

Olive oil production can be grouped under two topics as olive processing and olive oil storage.

2.2.1. Olive Processing

There are four important steps in olive oil processing; conditions prior to processing (olive picking, harvesting time and storage) and olive processing itself.

Picking of olive is usually done by hand or mechanical devices but picking up by hand is better to obtain undamaged olives. Damaged olives are in low quality and more prone to microbial attack. Harvesting time is crucial due to its effect on olive oil quality, stability, yield and sensorial characteristics. Early harvesting causes bitter and pungent taste while late harvesting results in undesired sweet taste; therefore, end of autumn or the beginning of the winter is generally regarded as the optimum harvest

time. After picking, there is a preliminary washing step which is used to remove any foreign materials (leaf, peduncle) in order to prevent damage to the machines and possible cross-contamination of the extracted oil (Baccouri et al., 2006; Di Giovacchino et al., 2002; Harwood & Aparicio, 2000).

Storage of olives prior to production is an important issue which should be kept under-monitoring. Actually, the collected olives should be transported into olive oil plant immediately to get high quality olive oil; however, most of the time this is not possible due to the low production capacity of the plant and some problems in production flow chart. Bad storage conditions like high temperature and inadequate air-conditioning provide excellent environment for bacterial growth causing low profile olive oil.

Olive processing consists of milling, malaxation, extraction and separation steps. Miiling step, based on crushing of olive, produces paste which is mainly comprised of oil droplets. Then, olive paste mixing stage as known as malaxation is used to break oil/water emulsion. In this part, mixing time and temperature are two important parameters that affect olive oil yield and quality. Main aroma compounds (volatiles) form at this stage and mainly affected by temperature; therefore, optimum mixing time and temperature are important and mixing time can be adjusted to 20-30 minutes and temperature of the paste should not exceed 25 °C (Angerosa et al., 2004; Aparicio & Harwood, 2000).

The slurry paste is produced at the end of malaxation and, extraction step is compulsory in order to obtain oil from paste. Extraction of olive oil is achieved by different methods like traditional pressing, modern centrifugation methods and percolation system.

Traditional press is a discontinuous system based on density differences between vegetable water and oil which is separated by resting liquid mixture in a series of tanks (Aktas et al., 2001).

Continuous system is an on-line process based on using decanter for extraction. It is grouped with respect to phase number as two or three phase system decanter. In a three phase system decanter; the addition of warm water is necessary to move the paste. Centrifugation force causes separation of olive oil and vegetation from oil pomace because of differences in weight. Centrifuge is used for further separation of olive oil and vegetation water (Harwood & Aparicio, 2000). Two phase system decanter is

superior over three-phase due to low energy and water usage. The only difference from three-phase system is obtaining two phases as oil and sludge (Caputo et al., 2003).

The last extraction method is percolation method which is based on usage of surface tension differences between oily part and vegetation water.

2.2.2. Storage of Olive Oil

Storage conditions of olive oil are of great importance to preserve its organoleptic properties and nutritional aspects. Under best storage conditions (optimum temperature, darkness etc.) olive oil can be kept without any spoilage for a long time but it is advised to be consumed in a few months. Long shelf life of olive oil is attributed to its high content of phenolic compounds, tocopherol, ratio of monounsaturated fatty acids and carotenoids

There are mainly four factors affecting olive oil quality during storage; packaging material, interaction with light, temperature and exposure to oxygen. Packaging material must be non-reactive and prevent light exposure as much as possible. These criteria could be obtained by dark brown glass material, ceramic, porcelain or stainless steel. In industry the main material used for olive oil storage is dark glass bottle (Caponio et al., 2005; Vekiari et al., 2007).

Light has a trigger effect in oxidation reaction which eventually results in deterioration of olive oil; therefore, olive oil must be stored in a cold environment without light.

2.3. Geographical Labelling

Some certain criteria were established by legal authorities in order to protect olive oil uniqueness and high quality which is also valid for other certain food stuff. EU Council is the leading organization in the world contributing new dimensions to quality known as Protected Designation of Origin (PDO) and Protection of Geographical Indication (PGI). PDO simply means that the food product is produced, processed, and prepared within the defined geographical region while PGI slightly different than the PDO implies that at least one of the steps mentioned above must occur in the specified geographical area. It is a growing sector all over the world due to the demand of

consumers for high quality products. For example, based on average of three years (2006-2008) data geographically labelled olive oil marketing value was determined as € 215 million per year in Europe according to European Commission (2012).

2.4. Olive Oil Chemical Composition

Chemical composition of olive oil consists of two main groups; major components as mainly triacylglycerols and small amounts of diacyglycerols, monoacylglycerols and free fatty acids. Minor components, on the other hand, comprise several numbers of heterogeneous compounds. According to Boskou (1996) these components are grouped under two main headings as non-chemically related to fatty acids and fatty acid derivatives. Non-chemically related ones are hydrocarbons (mainly squalene), alcohols, volatile compounds and antioxidants (tocopherol, pigments (carotenoids, chlorophylls) and phenolic compounds (Riachy et al., 2011a).

2.4.1. Major Components

Major components correspond to approximately 98% of olive oil in weight and are also known as saponifiable part. It is mainly composed of triacylglycerols (TAGs) whose structure is given in Figure 2.1. In addition, fatty acid groups are also formed by losing defined numbers of fatty acid from TAGs structure. If TAGs loose one fatty acid, it becomes diacylglycerol (DAGs) and if two fatty acid groups are removed, it is called as monoacylglycerol (MAGs). The released fatty acid becomes free fatty acid.

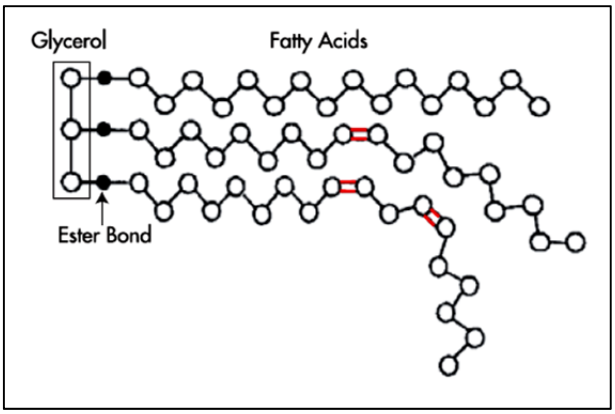

Figure 2.1. Triacylglycerol (oil) molecule with three different fatty acids attached. (Source: NSW Department of Primary Industries 2006)

The number of carbon atoms determines the chemical and physical characteristics of fatty acids. For example, four carbon atom containing fatty acid, butyric acid, is volatile while sixteen carbon atom molecule, palmitic acid, is solid at room temperature or oleic acid with eighteen carbon atom and with one double bond is liquid at room temperature.

According to bond structure, fatty acids can be grouped into two categories; unsaturated and saturated. Saturation stands for the entire carbon atom in fatty acids attached by single bonds. Unsaturation means at least one double bond exists in the molecular structure. It is also further divided in terms of number of double bonds as mono- has one double bond joining two carbon atoms and poly- have more than one double bonds joining carbon atoms. Bending position is named as -cis or -trans. Oleic acid being the main monounsaturated fatty acid in olive oil should be specifically emphasized due to its health contribution to human health and its chemical structure is shown in Figure 2.2 with other important fatty acids.

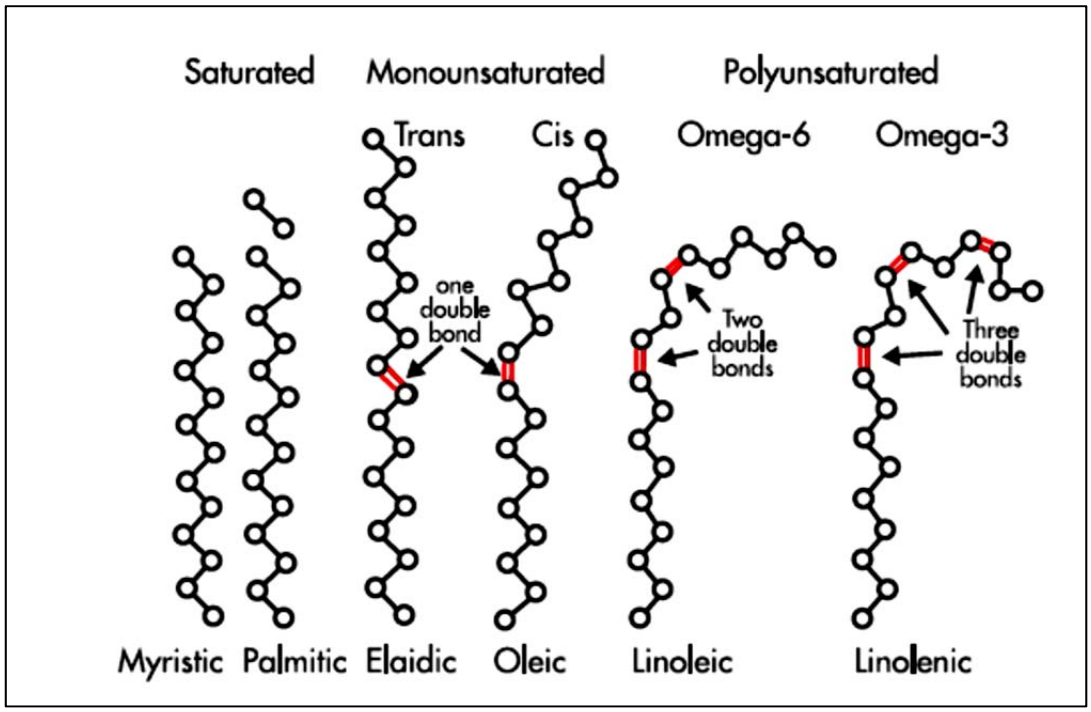

Figure 2.2. Structure of some important fatty acids in olive oil
(Source: NSW Department of Primary Industries 2006)

Being the most important part of olive oil, fatty acids, are also main parameters defining the olive oil quality. Firstly, olive oil is categorized in more general aspect in terms of free fatty acid content. Second, a quality criterion is also based on quantitative detection of some major fatty acids that exist in olive oil (Table 2.2).

Extra virgin olive oil: The oil which has free fatty acidity (FFA) ≤ 1.0 g per 100 g olive oil in terms of oleic acid according to EU (1991) and Turkish Food Codex (2000). IOOC (2014) has more strict rules on extra virgin olive oil and it is defined as FFA ≤ 0.8 g per 100 g olive oil.

Virgin olive oil: It is also known as natural first and criterion is FFA ≤ 2.0 g per 100 g olive oil which is agreed by all organizations (EU, Turkish Food Codex and IOOC).

Ordinary virgin olive oil (aka natural second): FFA ≤ 3.3 g per 100 g olive oil.

Virgin olive oil not available for consumption as it is named as lampante virgin olive oil: ≥ 3.3 g per 100 g used for refining or technological use.

Refined olive oil: It is the oil produced from virgin oil with refining, FFA ≤ 0.3 g per 100 g olive oil for both Turkish Food Codex and IOOC. For EU, FFA value must not be more than 0.5 g per 100 g.

Riviera olive oil: It is the mixture of refined olive oil and natural olive oil and FFA value ≤ 1.5 g per 100 g for Turkish Food Codex. EU and IOCC are in agreement with more strict value of FFA ≤ 1.0 g per 100 g.

Pomace oil: It is the oil which is extracted from olive pomace by refining methods like using solvent or other physical treatments which do not alter triglyceride structure of raw pomace oil. It is divided into two; refined pomace oil and mixed pomace oil.

Refined pomace oil: It can be found on market as its original state or mixed with natural olive oil and FFA ≤ 0.3 g 100 g olive oil (Turkish Food Codex, 2000; IOCC, 2014).

Mixed or olive pomace oil: It is the blend of refined olive pomace with virgin olive oil and FFA ≤ 1.5 per 100 g with respect to Turkish Food Codex and FFA ≤ 1.0 g per 100 g for IOOC.

Table 2.2. Fatty acid criteria of olive oil pomace, pomace oil and extra virgin olive (Source: Turkish Food Codex 2000, IOOC 2007, EU 1991)

	Olive and olive-pomace oil (Turkish Food Codex 2000, IOOC 2007)	Extra virgin oil (EU 1991)
Myristic acid (C14:0)	≤ 0.05	≤ 0.1
Palmitic acid (C16:0)	7.5-20	-
Palmitoleic acid (C16:1)	0.3-3.5	-
Margaric acid (C17:0)	≤ 0.3	-
Margoleic acid (C17:1)	≤ 0.3	-
Stearic acid (C18:0)	0.5-5.0	-
Oleic acid (C18:1)	55.0-83.0	-
Linoleic acid (C18:2)	3.5-21.0	-
Linolenic acid (C18:2)	≤ 0.9[1]	≤ 0.9
Arachidic acid (C20:2)	≤ 0.6	≤ 0.7
Eicosenoic acid (C20:1)	≤ 0.4	-
Behenic acid (C22:0)	≤ 0.2[2]	≤ 0.3
Lignoceric acid (C24:0)	≤ 0.2	≤ 0.5

[1] IOOC states this value ≤ 1
[2] This value of olive-pomace oil should be ≤ 0.3

2.4.2. Minor Components

2.4.2.1. Fatty Acid Derivatives

2.4.2.1.1. Sterols

Sterols are the most abundant part of non-saponificable fraction of lipids also known as phytosterols which have nutritionally greater importance. Content and composition of this fatty acid derivative depend on many factors like agronomic and climatic conditions, fruit quality and technological factors like oil extraction and refining conditions as well as end product storage conditions. These compounds are chemically very similar to cholesterol. Stigmasterol and sitosterol are dominant compounds found in phytosterols of crude olive oil. According to European Union Commission (2003), compositional analysis of the sterol fraction of olive oil not only provides an idea about its purity but also making it possible to define olive oil type (Canabate-Diaz et al., 2007). Phytosterols are also known for their cancer prevention mechanism with other secondary metabolites.

2.4.2.1.2. Phospholipids

There are many types of phospholipids that exit in olive oil and most important ones are phosphatidylcholine, phospatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol and phosphatidic acid among them. Cloudiness and oxidative stability of olive oil is affected by the presence of these mentioned compounds (Velasco & Dobarganes, 2002). Phospholipids may either act as antioxidant or pro-oxidant under certain conditions depending on their concentrations and the presence of certain metals like Fe^{+2} (Choe and Min, 2006).

2.4.2.1.3. Waxes

Waxes are the compounds which cover the skin of the fruit to prevent water loss form the system and they are esters of long chain aliphatic alcohols.

2.4.2.2 Non-Chemically Related Fatty Acid Compounds

2.4.2.2.1. Squalane

It is the most abundant hydrocarbon in olive oil which comprises nearly half of the unsaponifiable part. Its carcino-protective affect is detected against certain cancer types. In dark conditions, it can be transformed into α-tocopherol which is attributed to its antioxidant effect (Manzi et al., 1998).

2.4.2.2.2. Volatile Compounds

Volatile components are of great importance due to both their positive contribution to aroma and also sensorial defects of olive oils. Therefore, the presence or absence of definite volatiles can be a signature of high olive oil quality. These compounds produced by lipoxygenase pathway are known for their positive aroma contribution while chemical oxidation and some exogenous enzymes are responsible for aroma defects. Both the fruit and oil storage conditions and processing type affect severely quality parameters and flavor of olive oil (Angerosa, 2002; Kalua et al., 2007; Venkateshwarlu et al., 2004).

Volatile compounds can easily vaporize at room temperature and they have low molecular weights. Major aroma compounds of olive oil are aldehydes, alcohols, esters, hydrocarbons and ketones. Volatiles are mainly affected by cultivar type, geographical location of olive growth, ripening stage and technological factors. In detail, the aroma compounds are uprising with increasing ripening stage until a certain time. Post–harvest conditions for both olive and olive oil could cause different flavor perceptions. Technological factors, especially; the malaxation time and temperature have severe effect on flavor (Kalua et al., 2007).

The positive aroma perceptions are explained as fruity, bitter and pungent while sensorial defects are explained as fusty, musty-humid, muddy sediment, winery-vinegary, metallic and rancid.

2.4.2.2.3. Antioxidant Compounds

2.4.2.2.3.1. Color Pigments

Chlorophylls and carotenoids are lipophilic pigments which give the color of olive oil. Color is an organoleptic property which is really effective on consumer perception. Chlorophyll and carotenoid fractions of olive oils are affected by storage conditions, technological factors, degree of fruit maturity and geographical location. During ripening the concentration of chlorophyll decreases more drastically than carotenoids. Color pigments are also responsible for olive oil stability. It was pointed out that these compounds behave as antioxidants when olives were stored in dark conditions and they could act as pro-oxidants depending on their concentrations while under the light (Mateos et al., 2006; Roca et al., 2003).

2.4.2.2.3.2. Phenolic Compounds

2.4.2.2.3.2.1. Lipophilic Phenols (Tocopherols)

Lipophilic phenols are heteroacids of high molecular weight and α-tocopherol is the most abundant one of approximately 90% of tocopherols. There are other forms that also exist as β-tocopherols and γ-tocopherols. They are known for not only their antioxidant effect on human health but also exerting synergistic effect with other phenolic compounds to oxidative stress (Beltran et al., 2005; Mateos et al., 2003; Riachy et al., 2011a).

2.4.2.2.3.2.2. Hydrophilic Phenols

Phenolic acids, phenolic alcohols, hydroxyl-isochromans, flavonoids, secoiridoids and lignans which are shown in Figure 2.3 are important hydrophilic phenols. Their existences play an important role in olive oil quality due to their antioxidant activity, contribution on organoleptic properties and improvement of the shelf-life of the product. Polyphenols are defined as substances which possess a benzene

ring attaching one or more hydroxyl group, including functional derivatives (Carrasco-Pancorbo et al., 2005c).

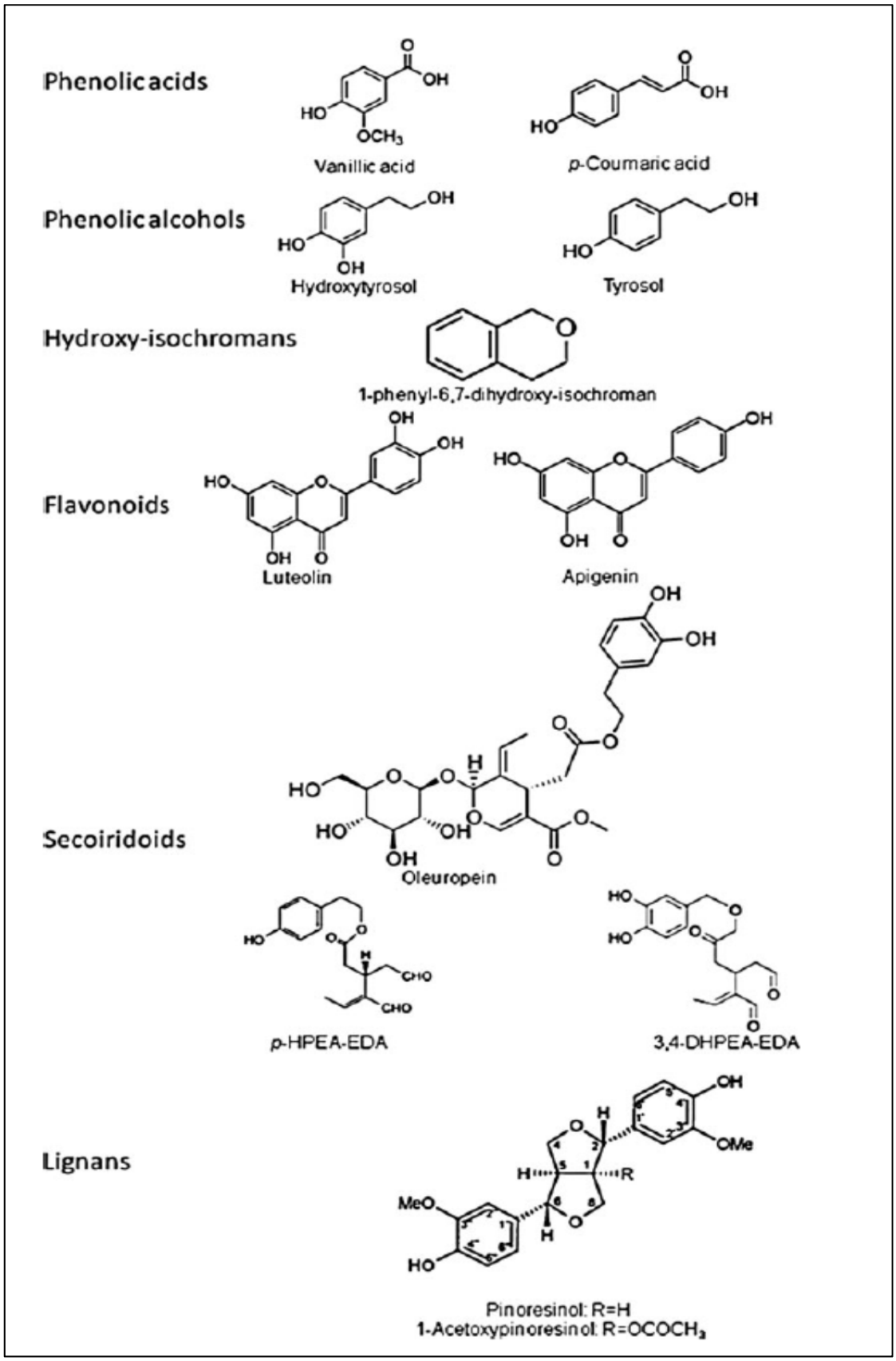

Figure 2.3. Structures of some important hydrophilic compounds in olive oil (Source: Riachy et al., 2011a)

Phenolic acids are the products of secondary metabolites which are responsible for color and sensorial attributes of olive oil. Major phenolic acids in olive oil are vanillic and p-coumaric acids. Moreover, these compounds could reveal a geographical effect on olive oil; therefore, they can be used in classification purposes (Buiarelli et al., 2004; Carrasco Pancorbo et al., 2005a).

Main phenolic alcohols of olive oil are hydroxytyrosol (3, 4-DHPEA) and tyrosol (*p*-HPEA). These compounds are the building blocks of secoiridoids; therefore, their concentration increases during storage of olive oil due to hydrolysis of secoiridoids (Brenes et al., 2001).

The most abundant phenolic compounds in olive oil are secoiridoids. They are mainly characterized by existence of either elenolic acid or its derivatives in their molecular structure. Major secoiridoids in olive oil are 3, 4- dihydroxyphenyl-ethanol (3, 4-DHPEA-EDA), p-hydroxyphenyl-ethanol (p-HPEA-EDA) and isomer of the oleuropein aglycone (3,4-DHPEA-EA). Moreover, oleuropein and ligstroside aglycone are other important components which were also grouped as secoiridoids (Carrasco-Pancorbo et al., 2005c; Owen et al., 2000a).

Flavonoids as the name implies are responsible for aroma occurrence with volatile compounds. Luteolin and apigenin are the most recognized flavonoids in olive oil.

Polyphenols are effective on olive oil stability as well as beneficial for human health by sustaining chemo-protective effect. Both of these effects can be attributed to antioxidant potential of phenolic compounds in olive oil. Chemo-protective effect can be defined as its protection against chronic and degenerative diseases; coronary heart diseases, neurological diseases and tumor formation. Moreover, they are also protecting low density lipoprotein (LDL) from damage of oxidative stress, decreasing the damage of the human erythrocytes and free radical occurrence in feces of human (Carrasco-Pancorbo et al., 2005c; Franceschi et al., 1999; Hodge et al., 2004; Manna et al., 1999; Owen et al., 2000b; Soler et al., 1998; Visioli et al., 1995).

There are many factors affecting the phenolic composition of olive oil because of complex formation mechanism of polyphenols. This mechanism is triggered by interactions of many factors like genotype, agronomical, environmental and technological conditions. To sum up, factors can be detailed as agronomic and environmental factors; maturity index of olive fruit, cultivation zone, water availability, sanitary state of drupes, alternate bearing in olive trees, and technological factors;

mainly milling and malaxation, and genetic variability of antioxidant; genetic variability between cultivars, genetic variability in wild olive trees, genetic variability in segregated population (Riachy et al., 2011b).

2.5. Analytical Methods for Geographical Origin

Demand to determine the origin food products are increasing day by day due to many reasons like organoleptic qualities attributed to regional products, health concerns, popularity on media, suspicious approach to the products from outside of their region (Gilg et al., 1998; Ilbery & Kneafsey, 1998; Kelly, 2003; Luyx & van Ruth, 2008).

There are lots of different analytical approaches in authentication studies and they can be mainly grouped into four categories as mass spectroscopy techniques, spectroscopic techniques (IR spectroscopy etc.), separation techniques (HPLC, GC etc.), and others (DNA technology etc.) (Table 2.3). Each of these techniques focuses on different constituents of olive oil; individually or combination of them like organic constituents, mineral contents, light or heavy element isotope ratio etc. Collected data using these techniques form a huge data cluster and needs chemometric analyses to interpret the results. If the targeted components or spectra have sufficient discriminatory power the results can be used in order to have a finger-print of the defined food-stuff belonging to specified geographical region (Luyx & van Ruth, 2008).

Table 2.3. Overview of analytical methods used for detection of geographical origin of food products (Source: Luyx & van Ruth, 2008)

Principle	Main technique	Specific forms of the technique
Mass spectrometry	Isotope ratio mass spectrometry (IRMS)	Continuous flow IRMS (CF-IRMS)
	Inductively coupled plasma mass spectrometry (ICP-MS)	Dual inlet IRMS (DI-IRMS)
	Proton transfer reaction mass spectrometry (PTR-MS)	
	Gas chromatography mass spectrometry (GC-MS)	
Spectroscopy	Nuclear magnetic resonance spectroscopy (NMR)	Low resolution NMR
		High resolution NMR (e.g., site specific natural isotope fractionation (SNIF))
	Infrared spectroscopy (IR)	Fourier transform IR (FTIR)
		Mid-infrared IR (MIR)
		Near-infrared IR (NIR)
	Fluorescence spectroscopy	Front-face fluorescence spectroscopy
	Atomic spectroscopy	Atomic absorption spectroscopy (AAS)
		Atomic emission spectroscopy (AES)
Separation	High performance liquid chromatography (HPLC)	
	Gas chromatography (GC)	
	Capillary electrophoresis (CE)	
Others	Sensor technology	'Electronic nose'
	DNA technology	Polymerase chain reaction (PCR)
	Sensory analysis	

In more general respect, the analytical methods for authentication studies can be also divided into two main categories like targeted analysis; based on detection of desired information from the fractionation of olive oil components, and the other approach is called profiling or non-targeted analyses which aim to identify general view of molecular structure based on predefined metabolic pathways (Aparicio et al., 2013; Baeten et al., 2005; Rezzi et al., 2005).

As one example of the profiling approach, IR spectroscopy does not have ability to elucidate analytes and rather focus on quick determination of genuineness of olive oils by screening many constituents at a time. These methods are gaining popularity on targeted approaches like GC and HPLC which are time-consuming and hardly producing perfect markers for classification (Aparicio et al., 2013).

All these approaches have their own advantages and disadvantages which are given in Table 2.4. Possible way to overcome the weakness of each methods could be combining them in together to increase mapping power of analytical methods. In the following sections, brief information and studies about separation techniques like HPLC and GC, and one of the spectroscopic methods, IR profiling, will be discussed.

Table 2.4. Advantages and disadvantages of analytical methods that are used in olive oil classification (Source: Luyx & van Ruth, 2008)

Techniques	Sensitivity	Simplicity	Time analysis	Costs	Reported applications	Compounds	Identification/profiling
MS							
IRMS	+	+/–	+/–	–	+	Various	i + p
ICP-MS	+	+/–	+	–	+	Elements	i + p
PTR-MS	+	+	+	–	–	Volatile	p
GC-MS	+	+	+/–	–	+	(Semi) volatile	i + p
Spectroscopy							
NMR	–	+/–	+/–	–	–	Various	i + p
IR	+/–	+	+	+	+	Various	p
Fluorescence	+	+	+	+	–	Various	p
Atomic	+/–	+/–	+/–	+/–	+	Elements	i + p
Separation							
HPLC	+/–	+	+/–	+	+	Various	p
GC	+	+	+/–	+	+	(Semi) volatile	p
CE	–	+	+/–	+	–	Various	p
Other							
Sensor technol.	–	+	+	+/–	+/–	Volatile	p
PCR	+	+/–	+/–	+	–	DNA	i + p
Sensory analys.	+/–	+/–	–	–	–	Various	p

The characteristics include the sensitivity and simplicity of the analytical tool, time of the analysis, instrumental costs, number of reported applications for determining the geographical origin, types of compounds which are analysed and possibility of identifying (i) and/or profiling (p) (including chemometrics) these compounds. Favourable (+), moderate (+/–), unfavourable (–).[a]

2.5.1. Gas chromatography (GC)

GC is one of the most popular techniques to separate volatile, semi-volatile, aromas and pesticides in food analysis (Chang et al., 1995; Luyx & van Ruth, 2008). Working principle is briefly based on injection of mixture of compounds into the GC where it is readily vaporized. Then, the gaseous mixture decomposes while travelling along GC column due to contact with the coating of the column. Eventually the separated constituents are detected by GC detector which produce electrical signal according to analyte amount exiting from the column (Grob, 2004).

In the literature, there are many examples of usage of GC to determine geographical origin of olive oils. Ollivier and co-workers (2003) characterize French olive oils by evaluating triacylglycerol and fatty acid compositions of the samples. In another study, GC was used to analyze 1004 monovarietal and multivarietal Sicilian olive oils from 22 different cutlivars in terms of fatty acid profile (D'Imperio et al., 2007). In the study of Stefanoudaki and co-workers (1999), 105 virgin olive oils from two dominant cultivars of Cretan; Koroneiki and Mastoides were collected to see the effect of maturation stage and location on fatty acid composition. The results showed

that olive oil obtained from Koroneiki cultivar differentiated by lower concentrations of oleic acid and higher concentrations of linoleic and palmitic acids. Moreover, oils obtained form higher altitude possessed more monounsaturated fatty acids than the lower-altitude ones known for containing higher precentages of saturated fatty acids. Final result of the study was palmitic and palmitoleic acids level was effected positively with increasing altitude. GC methodology was also used to check hydrocarbons discriminatory power on virgin olive oil. For this purpose, 105 olive oils obtained from seven Extremaduran olive varieties were analyzed in terms of alkane alkene, and sesquiterpene content at three stages of maturity. The results were quite promising with the value of 90% correct classification according to variety of the samples (Bueno et al., 2005). Another application area of GC is to analyze triacylglycerols and sterol composition. In the literature, Diaz et al. (2005) tried to classify olive oils obtained from different parts of Spain with respect to sterol and triacylglycerol composition. Best classification was achieved when triacylglycerol data was used alone. Lastly, a team from Tunusia characterized two main Tunisian cultivars, Chemlali and Chétoui in terms of their volatiles content (Tena et al., 2007).

2.5.2. High Performance Liquid Chromatography (HPLC)

HPLC is one of the liquid chromatography type used to separate, analyze and quantify compounds which is in the form of solution carried by mobile phase to the column. The column behaves as a stationary phase and the differences in partitioning behavior of the different components in the mixture separated as a result of these properties. HPLC is used for measuring many compounds like carbohydrates, vitamins, amino acids, lipids, phenols etc. (Luyx & van Ruth, 2008).

In olive oil studies, this technique has been used for a long time. In Turkey, one research group characterized Aegean olive oils sampled from different locations by determining phenolic profiles by HPLC (Ocakoglu et al., 2009). In another study, phenolic composition of virgin olive oils was studied. This study revealed high potential of antioxidant and also polyphenol and tocopherol contents of olive oil as a classification tool (García et al., 2003). Another approach was based on using pigment content to determine olive oil authenticity. A study from literature examined chlorophyll and carotenoid pigment composition of 12 monovarietal virgin olive oils

during one year of storage. It was found that pigment ratio like chlorophylls/carotenoids and minor carotenoids/lutein remained stable, notwithstanding to the variety and degree of maturity. In the light of these information prediction model for varietial separationprovided promising results (Roca et al., 2003). A team from Italy worked on olive oil extracted from three different area; Sciliy, Umbria, and Molise in terms of chlorophyll and carotenoid pigments composition by reversed-phase HPLC. They concluded that specific pigment profile in olive oils could be used in the authenticity studies (Giuffrida et al., 2011). In another study, tocopherol content was quantified with HPLC with some other minor and major components in order to classify Tunisian and Sicilian olive oils with respect to their original cultivation area (Baccouri et al., 2007). In a study by Aranda and co-workers (2004), it was tried to be visualized the effect of triglyceride (TG), total and 2-position fatty acid composition on classification of virgin olive oil obtained from Cornicabra variety in comparison with other Spanish cultivars. Principal component analysis and discriminant analysis showed that TG variables were more effective for the classification than total and 2-position fatty acids. Ultimate results of the study showed that many combinations of TGs and total fatty acid variables were available for successful classification.

2.5.3. Infrared Spectroscopy (IR)

This method depends on molecular vibrations created by each functional groups of a chemical compound and each group have unique vibrational frequency in the molecular structure under infrared absorption. When all the molecular structure is mapped in terms of wavenumber unique molecular fingerprint can be used as a molecular identity of a sample. IR spectrum is divided into three regions as near-, mid- and far infrared with respect to their visible spectrum range. IR spectroscopy is superior over other classical chromatographic methods (HPLC, GC etc.) since it is a short and an easy analysis technique. In addition, it is a non-invasive and a non-destructive method (Karoui et al., 2004). Especially; Fourier transform technique increases the usage of IR spectroscopy in food analysis. This technique sustains rapid screening and quantification of constituents (McKelvy et al., 1998).

With respect to mid-infrared and near-infrared profiles there are various studies in the literature aiming at different purposes. Mid-infrared profile was used to detect

geographical location of olive oil samples from Aegean region as well as detection of adulteration in olive oil samples in two different works of Gurdeniz et al. (2008, 2009). With NIR the geographical classification of extra virgin olive oils from Eastern Mediterranean was achieved by Downey and co-workers (2003). In another study, 60 oils from four European countries were used to analysis classification power of Fourier transform infrared spectroscopy (FTIR). Partial least squares distance-based linear discriminant analysis (PLS-LDA) was used to evaluate FTIR spectral data and genetic algorithm distance-based linear discriminant analysis (GA-LDA) approach was also used as a data analysis technique. PLS-DA was successfull at a rate of 96% cross-validation while GA-LDA was completely succesfull at a rate of 100% cross-validation (Tapp et al., 2003). Concha-Herrera and co-workers (2009) used FTIR in combination with chemometric techniques to classify seven different extra virgin olive oil varieties. LDA model correctly classified 88% of the olive oil (Concha-Herrera et al., 2009). FTIR has also been used in authentication of olive oils mixed with different types of oils. As a result of that study adulteration of virgin olive oil with hazelnut oil was detected at levels of 25% and higher (Ozen & Mauer, 2002).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Olive Oil Samples

The olive oil samples were obtained from the same olive cultivar, Erkence. Samples were from the various parts of Karaburun Peninsula as listed in Figure 3.1. 58 olive oil samples out of 64 were produced in the olive oil plant in Izmir Institute of Technology which has two phase decanter system for olive oil extraction and the rest were obtained from olive oil plant in Eğlenhoca Village, Karaburun having the same extraction system. All samples belonged to 2012-2013 harvest year and coded as in Table 3.1. Head space of the samples was flushed with nitrogen before storage and the samples were kept in the dark at refrigeration temperature (8 °C).

Figure 3.1. Olive oil samples from Karaburun Peninsula and its vicinity (Source: Google Earth 2013)

Table 3.1. Sampling locations, sample codes and number of samples from each location

Sample Location	Sample Code	Number of Samples
Barbaros	BR	4
Eğlenhoca	EH	7
Gülbahçe	GB	6
Karapınar	KP	3
Karareis	RS	2
Kuşcular	KS	5
Özbek	OZ	12
Torasan	TR	10
Urla	UR	5
Unknown	UK	10

3.1.2 Chemical Reagents

Reagents used in the experiments were analytical grade and obtained from Riedel-de Haën, Sigma-Aldrich and Merck. In HPLC analysis, phenolic acids; vanillic, syringic, caffeic, p-coumaric, o-coumaric, cinnamic, 4-hydroxyphenyl acetic, 3-hydroxyphenyl acetic and 2-3 dihydroxybenzoic acids and flavonoids; apigenin, luteolin, and vanilin and phenolic alcohols; tyrosol and hydroxytyrosol were used as commercial phenolic standards (Fluka and Extrasynthase) and fatty acid methyl ester (FAME) mixture containing C4-C24 (2-4% relative concentration) were used as a reference standard (Supelco # 47885-U) for GC analysis.

3.2. Methods

3.2.1. Oxidative Stability

Oxidative stability was measured by Rancimat equipment (873 Biodiesel, Metrohm, Switzerland) in terms of hour. Temperature range of this equipment is 50-220 °C and temperature stability is less than 0.1 °C. 3 g of olive oil was placed inside the glass reaction vessel. Carrier medium was selected as deionized water and it was used to fill the glass measuring vessel up to 60 mL. Reaction temperature was set to the constant value of 120 °C for both columns of Rancimat apparatus with constant 20 L/h air flow. In order to avoid any contamination which could cause the inaccurate readings, solvent resistant parts like reaction glass and glass measuring vessels were washed with acetone and detergent then rinsed with deionized water between each run while the fragile parts like O-ring and connection spacers rinsed with deionized water without any solvent. Heat resistant glassware parts were dried in an oven at 80 °C for 2 hr while heat sensible parts were left at room temperature for drying.

3.2.2. Total Phenol Content (TPC)

Modified Folin–Ciocalteu spectrophotometric method was used to determine the total phenolic compounds in the olive oil samples (Montedoro, et al. 1992). All the

results were calculated in terms of gallic acid (GA) as mg GA/kg oil. The measurements were repeated for two times for the extracted samples and three times for the gallic acid standard curve.

3.2.2.1. Extraction Procedure

10 mL of methanol/water mixture (80:20 v/v) plus 3 drops of Tween 20 (surfactant) was added to 2 g of olive oil sample and mixed with a homogenizer (Heidolph–Silent Crusher M, Germany) at 25000 rpm for 1 min and then centrifuged at 9000 rpm for 10 min (Sigma-2-16KC Centrifuge, The United Kingdom). After centrifugation supernatant (methanolic extract) was collected in a tube. Procedure was repeated for two more times by only adding 10 mL methanolic water mixture into the precipitate and mixing it with a homogenizer at 25000 rpm for 1 min and then centrifuging at 9000 rpm for 10 min. At the end of three extraction steps all supernatants were collected in the same tube and total volume was recorded.

3.2.2.2. Folin-Ciocalteu Method

0.5 mL of the methanolic extract of the olive oil was diluted to 3 mL with deionized water. After that 0.25 mL of Folin-Ciocalteu reagent was added and it was waited approximately for 1 min. Then, 1 mL of Na_2CO_3 solution (15% g/mL) was added and diluted with 0.75 mL of deionized water and mixed with a vortex for 30 sec. The same procedure using 0.5 mL methanol/water mixture without any extraction step was used for the preparation of blank samples. After the samples had been mixed with a vortex, they were left in a dark place for 2 hours and TPC of the extract was determined by spectrophotometric measurement at 765 nm. Stock solution (25 mg GA/ 25 ml methanol) was used to prepare varying concentrations of gallic acid solutions of 0.01-0.09 mg/mL. Three replicates were prepared for each concentration. Then, 1 mL of Na_2CO_3 solution (15% g/mL) was added and diluted with 0.75 mL of deionized water and mixed with a vortex for 30 sec. All of the procedures above were carried out for blank containing only 0.5 mL of methanol/water mixture (80:20 v/v). After the standards had been mixed with a vortex, they were left in a dark place for 2 hours and absorbance was taken spectrophotometrically at 765 nm. Standard GA curve is provided

in Figure 3.2. TPC was calculated using GA calibration curve with the following equation 1:

TPC (mg GA/kg OO) = GA equivalent (mg/mL) * Volume of extract (mL) * 1000 / weight of the OO (g) (Eq. 3.1)

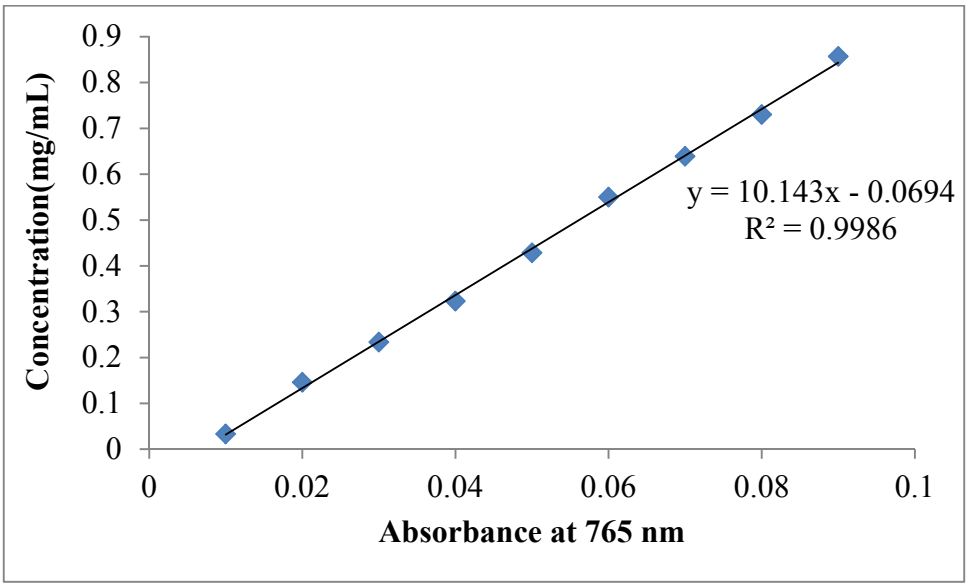

Figure 3.2. Standard gallic acid curve for TPC measurement

3.2.3. High Performance Liquid Chromatography (HPLC) Analysis of Phenolic Compounds

3.2.3.1. Extraction Procedure

The procedure from Brenes et al. (1999) was used to extract phenolic compounds from olive oil samples. Gallic acid solution as the internal standard was prepared by dissolving 0.05 g gallic acid (GA) in 25 mL methanol/water (80:20 v/v) mixture. This solution (1 mL) was added to olive oil sample before extraction. Extraction was done by adding 14 mL of methanol/water mixture into the internal standard-olive oil (14 g) complex and the solution was mixed with a homogenizer, and then centrifuged to separate the phases. Supernatant (phenolic extract) was collected in a beaker. Extraction step mentioned above was performed three more times with the precipitate occurred in each step. At the end of four extraction steps all of the supernatants were collected in the same beaker. Then, it was transferred into a round

bottom flask. To remove the methanol part from the system, a rotary evaporator (Heidolph Laborota-4000, Germany) was used at 35°C for 22 minutes under vacuum. After that 15 mL of acetonitrile was added to methanol free phenolic extract and then poured into the separation funnel to separate the phases by washing three times with 20 mL hexane. At the end of hexane wash step oily part was removed and the phenolic extract was poured into the round bottom flask again for further rotary evaporation under vacuum for 37 minutes at 35°C in order to remove acetonitrile from the phenolic extract. The residual part was flushed with nitrogen for approximately 10 minutes and dissolved in 1 mL of methanol/water. Finally, the extract was filtered through a 0.45 μm pore-sized membrane filter (Minisart, Sartorious, Goettingen, Germany) and transferred into dark brown HPLC vial and 20 μL of the extract was injected to HPLC system.

3.2.3.2. HPLC Analysis

Amounts of individual phenolic compounds in olive oil were determined by HPLC (Agilent 1200 HPLC, USA), equipped with refractive index (RI) and photodiode array (DAD) detectors, an auto sampler and a column oven. All the details about system specifications, analytical conditions and mobile phase are given in Table 3.2, Table 3.3, and Table 3.4.

Table 3.2. HPLC system specifications for phenolic profile determination of olive oils

System Specifications	
System	Agilent 1200
Detector Type	DAD
Automatic Sampler	ALS G1329A
Column	SGE 8211 C18 (250*4mm, 5 μm)

Table 3.3. Analytical conditions for HPLC determination of phenolic profile of olive oils

Analytical Conditions	
Column Temperature	35 °C
Injection Volume	20 µL
Flow Rate	1 mL/min
Mobile Phase, A	water/acetic acid (99.8:0.2 v/v)
Mobile Phase, B	Methanol
Wavelength	280, 320 nm

Table 3.4. Mobile phase concentration profile for HPLC measurement of phenolic profile of olive oils

Mobile Phase Concentrations		
Time (min)	% Mobile Phase, A	% Mobile Phase, B
0	90	10
10	70	30
30	70	30
40	60	40
45	60	40
50	50	50
55	50	50
60	40	60
65	30	70
70	0	100
85	90	10

Internal standard (ISTD) method was used in order to compensate any loss of phenolic compounds during the experimental procedures. Gallic acid was chosen as an internal standard due to its phenolic characteristic and it is not expected to be found in the olive oil samples. Major phenolic compounds found in olive oil like phenolic acids; vanillic, syringic, caffeic, *p*-coumaric, *o*-coumaric, cinnamic, 4-hydroxyphenyl acetic, 3-hydroxyphenyl acetic and 2-3 dihydroxybenzoic acids and flavonoids; apigenin, luteolin, vanilin and phenolic alcohols; tyrosol, hydroxytyrosol were determined by using their commercial standard forms. 5-point calibration curves for each standard were plotted. Finally, equations for each standard were used to evaluate the amount of phenolic compounds in terms of mg/kg found in olive oil. Standard curves are provided in Appendix B.

3.2.4. Chlorophyll & Carotenoid Measurement

Chlorophyll and carotenoid contents of olive oils were determined according to a procedure in literature (Mosquera et al, 1991). 7.5 g of an olive oil sample was weighted in a falcon tube and filled up to 25 mL with cyclohexane. The absorbances corresponding to chlorophyll and carotenoid fraction were measured by a UV spectrophotometer (Shimadzu UV-2450 Spectrophotometer, Japan) at 670 nm and 470 nm, respectively. The equations used for calculation of chlorophyll and carotenoid content are:

$$\text{Chlorophyll (mg/kg)} = Abs_{670} * 10^6 / 613 * 100 * D \quad (3.2)$$

$$\text{Carotenoid (mg/kg)} = Abs_{470} * 10^6 / 2000 * 100 * D \quad (3.3)$$

3.2.5. Fatty Acid Profile Determination

3.2.5.1. Sample Preparation

In order to determine fatty acid profile, first methyl esterification reaction was carried out. For this purpose, 0.1 g olive oil sample was weighted, then the samples were dissolved in 10 mL n-hexane and saponified to their methyl esters with the addition of 0.1 mL methanolic potassium hydroxide solution which had been prepared by dissolving 5.6 g potassium hydroxide in 50 mL methanol (EEC, 1991). The solution was vortexed for 30 s and centrifuged for 15 min at 5000 rpm. Supernatant was collected via syringe and filtrated into dark brown vials by using 0.45 μm syringe filter. Immediately after filtration impurity free supernatant was injected into the gas chromatography (GC) device.

3.2.5.2. Analytical Conditions

Fatty acid profiles of olive oil samples were examined by a GC (Agilent 6890, Agilent Technologies and Santa Clara, USA) equipped with an auto-sampler (Agilent

7863 & FID). The equipment specifications and analytical conditions are given in Table 3.5.

Table 3.5. System configurations and analytical conditions for GC determination of fatty acid profile of olive oils

System Configurations	
System	Agilent 6890 GC
Detector	FID
Automatic sampler	Agilent 7683
Column	100 m*0.25 mm ID, 0.2 μm HP-88 (J&W 112-88A7)
Inlet	Split/splitless
Liner	Split liner (p/n 5183-4647)
Analytical Conditions	
Inlet temperature	250°C
Injection volume	1 μl
Split ratio	1/50
Carrier gas	Helium
Head pressure	2 ml/min constant flow
Oven temperature	175°C, 10 min, 3°C/min, 220°C, 5 min
Detector temperature	280°C
Detector gases	Hydrogen:40 ml/min; Air:450 ml/min Helium make-up gas: 30 ml/min

3.2.6. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

All infrared spectra were recorded between the range of 4000-650 cm^{-1} wavenumber by Perkin Elmer Spectrum 100 FTIR spectrometer (Perkin Elmer Inc., Wellesley, MA) having a deuterated tri-glycine sulphate detector. The instrument was equipped with a horizontal attenuated total reflectance (HATR) accessory with ZnSe crystal. For each spectrum, the collected numbers of scans were 64 while the resolution was set to 4 cm^{-1} and scan speed was 1 cm/s. In between each run, crystal was cleaned with hexane, ethanol and deionized water. Measurements were repeated two times.

3.2.7. Statistical Analysis

All the statistical analyses were performed with SIMCA 13.0.3 software (Umetrics, Sweden). The need of multivariate evaluation exists due to chromatographic and spectroscopic methods' multivariate inheritance since more than one measurement can be made on a single sample (Brereton, 2003). In data analysis, whole FTIR spectra were used. Three multivariate methods were used as Principal Component Analysis (PCA), Soft Independent Modelling of Class Analogy (SIMCA) and Partial Least Squares (PLS) regression for data analysis in the present study.

3.2.7.1. Principal Component Analysis (PCA)

PCA is a well-known unsupervised multivariate technique used to decrease the number of observed variables into smaller number of artificial variables which explain the most of the variance in the data set. Eventually, data reduction is accomplished to create more meaningful interpretation from the data set (Stevens, 2012). Working principle of PCA is based on reducing the dimensionality of observed data to create new principal components (PCs) in which first PC explains the highest percentages of variation in the model after that second PC explains the second highest possible variation in the model and the number of PCs should be increased until the balance between degree of fit (R^2) and predictive ability (Q^2). Result of PCA can be given in two complementary plots as scores and loading plots. Score plot indicates how the observations are scattered and which of them are clustered to differentiate principal groupings among observations while loading plots are focused on variables to reveal which variables are responsible from the groupings among the observations (Euerby & Petersson, 2003). In the present study, classification studies were performed separately by using 19 fatty acids, 15 phenolic compounds with and without TPC, 3351 wavenumbers from FTIR spectra with raw form and modified (second derivate) forms as data sets. In addition to that, fatty acids, phenolic compounds and TPC, chlorophyll & carotenoid content, oxidative stability were used together as another data set.

3.2.7.2. Soft Independent Modelling of Class Analogy (SIMCA)

Coomans' plot is used to visualize the SIMCA results of two different classes to determine how well classifications occurred. Main idea of constructing Coomans' plot is to show the discrimination of two classes on the plot which is composed of four zones defined as class 1, class 2, overlap of both classes, and an outer space (distant from both classes). It is expected for each class to be located in their critical limits in order to be sure about the perfect classification.

3.2.7.3. Partial Least Squares (PLS) Regression

PLS is a supervised regression method which aims at predicting Y variables (fatty acid content MUFA, PUFA, SFA included, phenolic composition TPC included, chlorophyll & carotenoid content and oxidative stability) from X variables (MIR spectra) by maximizing the correlation between them. In order to increase the predictive ability of the PLS model second derivative of FTIR profile is used in order to eliminate noises and shifts (Eriksson et al., 2000). There are certain parameters to determine the wellness of prediction model like root mean square error of calibration (RMSEC) and cross-validation (RMSECV), regression coefficients for calibration (R^2 cal) and cross-validation (R^2 cv). In detail, regression coefficient gives an idea about the prediction efficiency and both calibration and validation R^2 must be close to one for a good model (Bauer et al., 2008). RMSEC and RMSECV values are related with the error between actual value and predicted value at each calibration step and cross-validation step, respectively. It is expected that differences between RMSEC and RMSECV value must be small and close to zero due to the fact that each of these values is attributed to the error; therefore, the main idea of good prediction is to minimize the error. Comparison of RMSEC and RMSECV values reveals whether calibration model is over-fitted or not (Muik et al., 2004). When evaluating the results of a prediction model all of these parameters must be taken into consideration. RMSECV value is calculated by SIMCA software. Equation for RMSEC:

$$\mathbf{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n-2}} \qquad (3.4)$$

In the formula n stands for the samples used in calibration set, y_i is the actual value of the sample while $\hat{y}_i$ is the predicted value.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Chemical Characterization of Olive Oils from Karaburun Region of İzmir

Karaburun Peninsula and its close surrounding area is a region where olive oil production is a significant economic activity for the local people. Erkence variety is almost the only significant olive variety grown in this region. Erkence type is mainly cultivated in İzmir, specifically Karaburun Peninsula and its vicinity and is also known as İzmir Yağlık which is mostly used for olive oil production due to its high olive oil content (~25%). In order to investigate the chemical characteristics of olive oils from Karaburun Peninsula region, several important chemical parameters like oxidative stability, total phenol content, chlorophyll and carotenoid content, fatty acid content, FTIR profile and composition of phenolic compounds were evaluated. Samples were divided into nine areas as Eğlenhoca (EH), Karareis (RS), Karapınar (KP), Barbaros (BR), Gülbahçe (GB), Torasan (TR), Kuşcular (KS), Özbek (OZ) and Urla (UR) according to the growth location of olives prior to data evaluation. There were total of 64 samples and the number of samples from each region as it is; EH = 7, RS = 2, KP=3, BR=4, GB=6, TR=10, KS=5, OZ=12, UR=5. There were also 10 samples which are from Karaburun region but their exact locations are unknown. Unknown origin samples were not used in classification study but they were used in PLS analysis part. Results of the chemical parameters for olive oils from Karaburun region are provided in Table 4.1-4.7.

4.1.1. Oxidative Stability

Oxidative stability (OS) of olive oils mainly depends on two compositional factors: fatty acid profile (type of fatty acid especially the number of double bonds) and the concentration of minor compounds (effective on stability despite their low concentration) like color pigments (chlorophyll & carotene), phenolic substances, tocopherol content and volatile components (Ceci & Carelli 2007). Compositional

parameters that determine OS are affected by the olive variety (genetic) and quality, geographical location (altitude, irrigation regime, soil conditions, climate etc.) and maturation stage (Dıraman et al., 2009 Gimeno, et al. 2002; Gutieraz, et al. 1999; Kamoun, et al. 2007; Laroussi, et al. 2006; Tawalbeh, et al. 2006,). Post-harvest conditions such as storage temperature, light and oxygen concentration altogether called as extrinsic factors are also influencing olive oil oxidation during storage period (Velasco & Dobargenes, 2002).

OS of all the olive oil groups used in this study are presented in Table 4.1 and values fluctuated between 0.1 and 4.41 h. It was observed that olive oils belonging to EH region have the highest average oxidative stability (3.36 h) while the rest of the samples have lower values which are close to each other.

In the present study, Rancimat method, one of the accelerated shelf life tests, was used to investigate the stability of olive oils against the oxidative stress. In the recent studies, there have been discussions about Rancimat method efficiency. According to Beŝter (2008) Rancimat provides the overall prediction about the antioxidant potential of the olive oil without giving any information about the possible contribution of single compounds which could be positive or negative in manner. In another study, it was stated that high temperature tests to predict OS are found to be questionable due to the changes in the mechanism of lipid oxidation at the elevated temperatures and this could mislead the prediction of the effectiveness of antioxidants (Frankel, 2010). Although there are some weak points of Rancimat, today it is still widely used in OS studies because sample preparation for the test is easy and results are obtained in a short time.

Table 4.1. Oxidative stability values of olive oils from Karaburun region

Location	Oxidative Stability (OS)		
	Mean	Range	
	(h)	Min	Max
Eğlenhoca (EH)	3.36±0.49	2.41	3.94
Karareis (RS)	1.60±1.17	0.77	2.43
Karapınar (KR)	1.64±1.39	0.10	2.80
Barbaros (BR)	1.89±1.96	0.16	4.41
Gülbahçe (GB)	1.86±1.52	0.37	4.35
Torasan (TR)	1.13±1.25	0.17	3.75
Kuşcular (KS)	1.14±0.35	0.65	1.52
Özbek (OZ)	1.13±0.56	0.37	2.19
Urla (UR)	1.79±1.67	0.16	3.92
Unknown (UK)	2.03±1.30	0.82	4.31

4.1.2. Chlorophyll & Carotenoid Content

Color pigments, chlorophyll and carotenoid were determined in the range of 0.51-4.53 mg/kg oil and 0.26-15.12 mg/kg oil, respectively (Table 4.2 and 4.3). Compared to data from literature color pigments of olive oils obtained from Erkence variety is lower than the olive oils obtained from Italian olive cultivars. In detail, it was found that Sicilian olive oil total chlorophyll amount fluctuates between 24.95-31.97 mg/kg while total carotenoid amount fluctuates between 18.32-27.44 mg/kg which was quantified by reversed-phase liquid chromotography (Giuffrida et al., 2007). In another study, researchers worked on olive oil samples obtained from southern and central Italy and total chlorophyll and carotenoid content of these oils were in the range of 1.00-26.64 mg/kg and 4.19-14.26, respectively (Giuffrida et al., 2011). The method used in the present study was based on traditional spectrophotometric technique which provides estimation about total chlorophyll and carotenoid contents. RS region olive oils had the highest average chlorophyll content (2.82 mg/kg) while KS region oils had the lowest (1.30 mg/kg). In terms of carotenoid content GB region was the highest (5.86 mg/kg) and the lowest one was TR region. No relation was established between the carotenoid

and chlorophyll contents of olive oils. Amounts of chlorophyll and carotenoid contribute to olive oil oxidative stability in a positive way under certain conditions. According to two follow up studies in literature, chlorophyll showed an antioxidant effect when the oil is stored in dark whereas in the presence of light the chlorophyll could behave as a pro-oxidant (favor the oxidation); therefore, it is really important to store products in the dark conditions to protect the organoleptic properties of the oils (Psomiadou and Tsimidou, 2002a & 2002b; Roca et al., 2003).

Table 4.2. Total chlorophyll content of olive oils from Karaburun region

Location	**Chlorophyll Content**		
	Mean	Range	
	(mg/kg)	Min	Max
Eğlenhoca (EH)	1.50±0.54	0.51	2.23
Karareis (RS)	2.82±1.94	1.45	4.19
Karapınar (KR)	1.76±0.83	1.00	2.64
Barbaros (BR)	2.07±1.23	1.02	3.86
Gülbahçe (GB)	2.45±1.03	1.15	3.58
Torasan (TR)	1.35±0.47	0.76	1.90
Kuşcular (KS)	1.30±0.36	0.83	1.57
Özbek (OZ)	1.94±1.09	1.04	4.53
Urla (UR)	2.45±1.21	1.25	4.16
Unknown (UK)	2.60±2.74	0.81	8.84

Table 4.3. Total carotenoid content of olive oils from Karaburun region

Location	Caretonoid Content		
	Mean	Range	
	(mg/kg)	Min	Max
Eğlenhoca (EH)	2.68±1.33	0.26	4.49
Karareis (RS)	5.13±5.41	1.31	8.96
Karapınar (KR)	4.48±1.70	3.36	6.43
Barbaros (BR)	4.50±7.12	0.38	15.12
Gülbahçe (GB)	5.86±3.09	2.41	9.27
Torasan (TR)	2.12±1.10	1.00	4.21
Kuşcular (KS)	2.81±1.91	0.73	5.19
Özbek (OZ)	4.39±2.98	1.16	9.58
Urla (UR)	5.81±4.37	0.31	10.38
Unknown (UK)	5.01±8.23	0.11	25.63

4.1.3. Fatty Acid Profile

Fatty acid profiles of all the olive oil samples are presented in Table 4.4 & 4.5 and typical GC chromatogram of one of the oils is shown in Figure 4.1. The distribution of fatty acids in olive oil samples is in the ranges of European Standard for Olive Oils and Olive Pomace Oils (EC 796/2002). The fatty acid values of the samples from different areas are quite close to each other. According to literature, fatty acid composition is mainly affected by genotype (cultivar) (Lanteri, et al. 2002). In this study, all the olive oil samples belong to the same olive cultivar (Erkence). The most abundant monounsaturated fatty acid (MUFA) that exists in the olive oil is oleic acid and KP region has the highest amount (70.45%) of this fatty acid followed by RS region (70.01%). There are very few studies in the literature about the Erkence type of olive oil and according to one of these studies olive oil from Erkence variety harvested in 05/06 and 06/07 season had oleic acid content of 66.44% and 63.57%, respectively (Gurdeniz, et al. 2008). It was observed that there existed a slight decrease between season 05/06 and 06/07 in terms of oleic acid percentages. In this study, the oleic acid content (68.92%) in terms of average sum of the whole Karaburun Peninsula region was higher compared to previous study. Other important polyunsaturated fatty acid (PUFA),

linoleic acid, is determined in the range of 7.87% to 15.13% with the average of value of 11.78%. Again in Gurdeniz et al. study (2008), the linoleic acid percentages were 14.95% and 16.89% in two different harvest seasons. All the fluctuations observed between the years could be attributed to the climatic conditions at different harvest years and differences in geographical locations of extracted oils.

MUFA and PUFA contents of the olive oils are important parameters for the prediction of OS of the olive oil. The MUFA content, especially the most abundant one, oleic acid, has positive effect on oxidative stability of oils while the PUFA content (linoleic + linolenic) are more susceptible to the oxidation than MUFA due to the number of double bonds (Dıraman, et al. 2009).The ratio of oleic/linoleic + linolenic acids (OLLnR) are used to have an idea about the contribution of fatty acid profile to OS. For the present study, the amount of saturated fatty acids (SFA), MUFA, PUFA and OLLnR are given in the Table 4.4. According to the table, MUFA content of RS region is slightly higher (71.94%) than the rest of the regions whereas UR region is the lowest. The higher OLLnR value is associated with the higher OS. The highest OLLnR value is 7.34 for RS region but unexpectedly the OS value for RS region is 1.6 h and lower than EH region; therefore, no linear correlation is established between OS and OLLnR (R^2 =0.13). This situation could be explained by the fact that OS is related not only with fatty acid composition but also with many other factors like pro- and/or anti-oxidant compounds (Ceci, et al. 2007).

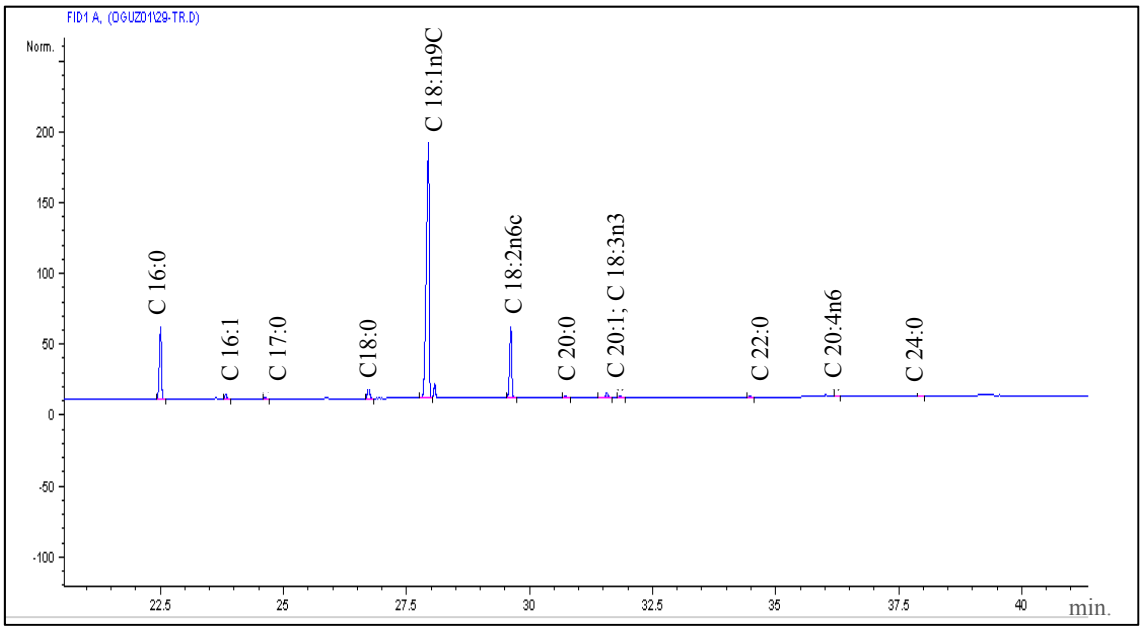

Figure 4.1. Sample GC chromatogram of olive oil from EH region

Table 4.4. General fatty acid composition (% of total fatty acids) of olive oils from Karaburun region

Location	SFA*			MUFA*			PUFA*			OLLnR*		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
	(%)	Min	Max	(%)	Min	Max	(%)	Min	Max	(%)	Min	Max
Eğlenhoca (EH)	15.25	14.25	16.01	70.27	68.24	72.94	14.43	12.82	15.47	4.79	4.63	5.27
Karareis (RS)	18.48	17.86	19.11	71.94	70.50	73.38	9.58	8.84	10.32	7.34	6.94	7.80
Karapınar (KR)	16.82	15.99	17.55	71.88	70.66	73.67	11.30	9.08	12.62	6.25	5.73	7.65
Barbaros (BR)	17.05	15.56	18.37	71.33	69.41	73.83	11.62	8.45	14.38	6.03	5.04	8.07
Gülbahçe (GB)	17.47	16.26	19.64	70.08	68.24	72.35	12.50	8.92	14.03	5.50	5.04	7.57
Torasan (TR)	17.54	16.28	19.31	69.87	67.13	73.44	12.60	8.15	14.82	5.42	4.80	8.06
Kuşcular (KS)	17.98	16.61	19.66	70.82	68.94	71.93	11.20	9.30	12.42	6.16	5.63	7.26
Özbek (OZ)	17.33	16.31	18.96	69.43	67.47	72.36	13.22	11.69	15.82	5.16	4.57	5.66
Urla (UR)	18.33	16.09	19.93	68.94	66.91	70.48	12.73	11.25	14.74	5.31	4.69	5.84
Unknown (UK)	16.94	13.51	19.04	72.07	68.90	78.61	10.99	4.90	14.35	6.42	5.35	13.85

SFA* : total saturated fatty acids, MUFA* : monounsaturated fatty acids, PUFA* : polyunsaturated fatty acids, OLLnR* : oleic/linoleic + linolenic acids

Table 4.5. Individual fatty acid content (% of total fatty acids) of olive oils from Karaburun region

Fatty Acids	Eğlenhoca(EH)			Karareis(RS)			Karapınar(KP)		
	Mean	Range		Mean	Range		Mean	Range	
	(%)	Min	Max	(%)	Min	Max	(%)	Min	Max
C8:0	nd	nd	nd	0.01±0.02	nd	0.02	0.01±0.02	nd	0.03
C14:0	0.01±0.01	nd	0.02	nd	nd	nd	0.01±0.01	nd	0.02
C15:0	nd	nd	nd	nd	nd	nd	nd	nd	nd
C16:0	11.73±0.54	10.88	12.38	13.86±0.45	13.54	14.18	13.06±0.52	12.46	13.42
C16:1	0.44±0.14	0.13	0.52	1.14±0.19	1.00	1.27	0.74±0.06	0.68	0.81
C17:0	0.14±0.00	0.13	0.15	0.18±0.04	0.15	0.20	0.12±0.03	0.09	0.14
C18:0	2.81±0.08	2.70	2.89	3.71±0.31	3.48	3.94	2.96±0.25	2.80	3.24
C18:1n9c	69.15±1.43	67.51	71.69	70.01±1.79	68.74	71.27	70.45±1.49	69.33	72.14
C18:2n6c	14.11±0.94	12.51	15.13	9.23±1.01	8.51	9.94	10.96±1.91	8.77	12.23
C20:0	0.43±0.01	0.42	0.44	0.51±0.04	0.48	0.53	0.45±0.01	0.44	0.47
C20:1	0.68±0.05	0.59	0.73	0.80±0.07	0.75	0.85	0.69±0.04	0.65	0.72
C18:3n3	0.32±0.01	0.31	0.34	0.31±0.03	0.30	0.33	0.32±0.03	0.29	0.35
C21:0	nd	nd	nd	nd	nd	nd	0.01±0.01	nd	0.02
C20:2	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:0	0.13±0.00	0.12	0.13	0.13±0.00	0.13	0.14	0.13±0.01	0.13	0.14
C20:3n6	nd	nd	nd	nd	nd	nd	nd	nd	nd
C20:4n6	nd	nd	nd	0.04±0.02	0.03	0.05	0.03±0.01	0.02	0.04
C24:0	nd	nd	nd	0.09±0.01	0.08	0.10	0.08±0.01	0.07	0.08

nd: not determined

(cont. on next page)

Table 4.5. (cont.) Individual fatty acid content (% of total fatty acids) of olive oils from Karaburun region

Fatty Acids	Barbaros(BR)			Gülbahçe(GB)			Torasan(TR)		
	Mean	Range		Mean	Range		Mean	Range	
	(%)	Min	Max	(%)	Min	Max	(%)	Min	Max
C8:0	0.01±0.01	nd	0.02	0.01±0.01	nd	0.02	0.01±0.02	nd	0.04
C14:0	nd	nd	nd	0.01±0.01	nd	0.02	nd	nd	nd
C15:0	nd	nd	nd	0.00±0.01	nd	0.03	nd	nd	nd
C16:0	13.21±0.47	12.60	13.70	13.67±0.91	12.92	15.20	14.01±0.49	13.41	15.07
C16:1	0.82±0.17	0.64	1.01	0.72±0.44	nd	1.35	0.91±0.19	0.76	1.42
C17:0	0.11±0.08	nd	0.16	0.14±0.03	0.12	0.19	0.13±0.05	nd	0.16
C18:0	3.07±0.40	2.62	3.57	2.97±0.20	2.73	3.30	2.74±0.18	2.49	3.09
C18:1n9c	69.81±1.72	68.16	72.07	68.57±0.93	67.52	70.02	68.14±1.75	65.69	70.70
C18:2n6c	11.31±2.56	8.21	14.01	12.14±1.81	8.67	13.46	12.23±2.02	7.87	14.26
C20:0	0.45±0.08	0.34	0.51	0.45±0.05	0.39	0.53	0.44±0.03	0.38	0.49
C20:1	0.70±0.07	0.60	0.75	0.80±0.10	0.71	0.97	0.82±0.19	0.69	1.32
C18:3n3	0.28±0.03	0.24	0.30	0.32±0.06	0.25	0.43	0.34±0.06	0.28	0.48
C21:0	0.02±0.04	nd	0.07	0.01±0.01	nd	0.02	0.00±0.01	nd	0.03
C20:2	0.01±0.02	nd	0.05	0.01±0.04	nd	0.09	nd	nd	nd
C22:0	0.12±0.10	nd	0.23	0.13±0.02	0.09	0.15	0.11±0.04	nd	0.14
C20:3n6	nd	nd	nd	nd	nd	nd	0.00±0.01	nd	0.04
C20:4n6	0.02±0.02	nd	0.04	0.03±0.01	nd	0.04	0.03±0.02	nd	0.04
C24:0	0.06±0.05	nd	0.11	0.08±0.06	nd	0.19	0.10±0.08	nd	0.30

(cont. on next page)

Table 4.5. (cont.) Individual fatty acid content (% of total fatty acids) of olive oils from Karaburun region

Fatty Acids	Kuşcular(KS)			Özbek(OZ)			Urla(UR)		
	Mean	Range		Mean	Range		Mean	Range	
	(%)	Min	Max	(%)	Min	Max	(%)	Min	Max
C8:0	nd	nd	nd	0.01±0.01	nd	0.03	0.01±0.02	nd	0.05
C14:0	0.01±0.01	nd	0.01	0.01±0.01	nd	0.03	nd	nd	nd
C15:0	nd	nd	nd	nd	nd	0.01	nd	nd	nd
C16:0	14.41±0.68	13.49	15.22	13.56±0.42	13.04	14.23	13.97±0.77	12.70	14.66
C16:1	1.16±0.23	0.82	1.41	0.76±0.11	0.64	0.93	0.76±0.15	0.58	0.98
C17:0	0.13±0.01	0.12	0.14	0.15±0.01	0.14	0.17	0.18±0.04	0.14	0.24
C18:0	2.76±0.38	2.42	3.42	2.96±0.35	2.62	3.70	3.40±0.06	2.70	3.94
C18:1n9c	68.91±0.90	67.50	69.70	67.88±1.04	66.19	70.00	67.39±1.21	65.66	68.56
C18:2n6c	10.89±1.14	9.06	12.06	12.80±1.03	11.40	14.49	12.37±1.48	10.96	14.24
C20:0	0.45±0.07	0.40	0.58	0.45±0.03	0.40	0.49	0.54±0.09	0.45	0.63
C20:1	0.75±0.08	0.62	0.82	0.79±0.21	0.64	1.44	0.80±0.10	0.67	0.95
C18:3n3	0.29±0.03	0.25	0.31	0.35±0.15	0.29	0.83	0.32±0.04	0.29	0.38
C21:0	0.00±0.01	nd	0.02	0.01±0.01	nd	0.04	0.01±0.01	nd	0.02
C20:2	nd	nd	nd	nd	nd	nd	0.01±0.02	nd	0.04
C22:0	0.11±0.01	0.10	0.13	0.13±0.01	0.11	0.15	0.15±0.03	0.11	0.18
C20:3n6	0.00±0.01	nd	0.01	0.01±0.02	nd	0.05	0.01±0.01	nd	0.03
C20:4n6	0.02±0.01	nd	0.03	0.06±0.12	nd	0.43	0.02±0.01	nd	0.03
C24:0	0.10±0.03	0.07	0.15	0.07±0.03	nd	0.12	0.09±0.07	nd	0.20

Table 4.5. Individual fatty acid content (% of total fatty acids)

Fatty Acids	Unknown (UK)		
	Mean (%)	Range	
		Min	Max
C8:0	0.01±0.02	nd	0.06
C14:0	0.01±0.01	nd	0.02
C15:0	nd	nd	nd
C16:0	13.00±1.08	10.35	14.25
C16:1	0.85±0.22	0.42	1.14
C17:0	0.15±0.01	0.13	0.17
C18:0	3.12±0.43	2.50	3.70
C18:1n9c	70.49±2.72	67.91	76.59
C18:2n6c	10.71±3.20	4.90	13.99
C20:0	0.46±0.04	0.40	0.53
C20:1	0.73±0.10	0.57	0.89
C18:3n3	0.27±0.10	nd	0.34
C21:0	0.01±0.01	nd	0.02
C20:2	nd	nd	nd
C22:0	0.13±0.01	0.12	0.16
C20:3n6	nd	nd	nd
C20:4n6	0.01±0.01	nd	0.03
C24:0	0.05±0.06	nd	0.14

4.1.4. Phenolic Composition

TPCs of all the locations are given in Table 4.6 and it was observed that the olive oils obtained from BR region possess the highest value of TPC with the mean of 327.42 mg GA/kg oil whereas KR region have the lowest (266.37 mg GA/kg oil). According to a study in literature, Erkence olive oil has TPC as 356.65 and 333.37 mg GA/kg oil in different harvest years (Ocakoglu, et al. 2009). In the present study, the average sum of the whole Karaburun Peninsula region's TPCs is determined as 277.67 mg GA/ kg which is lower compared to previous study. TPCs are affected by many factors like environment, harvest year and geographical location (Ocakoglu, et al. 2009).

Findings of the present study revealed that higher TPC does not always result in higher OS. When these two parameters are plotted against each other the correlation coefficient, R^2 (0.101), is very low. Therefore, no significant relation is established between TPC and OS. An example for this situation is EH region having one of the high OS values of 3.36 h while TPC of this region is one of the lowest (243.68 mg GA/ kg oil).

Table 4.6. TPCs of olive oils of from Karaburun region

Location	Total Phenol Content(TPC)		
	Mean	Range	
	(mg/kg)	Min	Max
Eğlenhoca (EH)	243.68±20.87	211.09	261.30
Karareis (RS)	238.56±60.51	195.77	281.34
Karapınar (KR)	266.37±35.72	226.46	295.35
Barbaros (BR)	327.42±57.49	285.39	409.98
Gülbahçe (GB)	290.12±106.12	209.90	491.95
Torasan (TR)	301.10±64.17	192.31	397.07
Kuşcular (KS)	260.60±31.62	214.28	295.11
Özbek (OZ)	288.05±67.37	225.98	474.74
Urla (UR)	283.11±62.74	237.83	392.28
Unknown (UK)	265.79±46.25	188.46	349.59

Phenolic composition of the extracted olive oils from different parts of Karaburun Peninsula were evaluated with HPLC analysis and a typical chromatogram of a phenolic extract is given in Figure 4.2 at two different wavelengths. Significant phenolic compounds for the studied region are hydroxytyrosol, tyrosol, 4-hydroxyphenyl acetic acid, 3-hydroxyphenyl acetic acid, vanillic acid, vanillin, p-coumaric acid and apigenin. Tyrosol was the most abundant phenolic compound in terms of average sum of the whole Karaburun Peninsula region (10.15 mg/kg olive oil). The second highest was hydroxytyrosol with a value of 4.56 mg/kg according to Table 4.7. Luteolin, syringic, cinnamic and caffeic acids were found in trace amounts whereas o-coumaric, 2-3 dihydroxybenzoic and chlorogenic acids were not determined for most of the regions or found in negligible amounts.

In a study from literature, Erkence olive oil was classified with other 5 cultivars (Memecik, Domat, Nizip-yağlık, Gemlik and Ayvalık) and all the cultivars were rich in terms of hydroxytyrosol, tyrosol, vanillic acid, p-coumaric acid, cinnamic acid, luteolin, and apigenin (Ocakoglu, et al. 2009). Erkence is the variety in the present study and 4-hydroxyphenyl acetic acid, 3-hydroxyphenyl acetic acid, and vanillin were also

abundant and were the different compounds from the past study with only exception of luteolin which was found in trace amounts. Hydroxytyrosol (1.43-7.71mg/kg) and tyrosol (4.11-16.39 mg/kg) were higher than the amounts detected in the previous study while apigenin, luteolin and cinnamic acid concentrations were really lower comparatively. 4-hydroxphenyl acetic, vanillic and p-coumaric acids were detected in significantly higher amounts. The rest of the phenolic compounds (2-3 dihydroxybenzoic acid, caffeic acid and vanillin) were in similar amounts with the previous work (Ocakoglu, et al. 2009).

Individual phenolic profile of the present study showed that BR region olive oils were the richest in terms of hydroxytyrosol and tyrosol (orderly, 7.71 and 16.39 mg/kg) than the rest whereas RS region possesed the lowest value of tyrosol (4.11 mg/kg) and 3-hydroxyphenyl acetic acid (0.35 mg/kg). This region oils also had the highest syringic acid concentration. TR region became prominent due to the highest value of 4-hydroxyphenyl acetic acid and 3-hydroxyphenyl acetic acid. KS region had the highest p-coumaric acid with the value of 2.39 mg/kg. EH region had the highest value of luteolin while the lowest value of apigenin.

In the literature, correlation between the individual phenolic compound and OS were investigated and a positive relation was found between the amount of hydroxytyrosol and OS value (Tura, et al. 2007). In the present study, all the individual phenolic compounds are also investigated in terms of their correlation with OS. No significant relation is determined between the amounts of hydroxytyrosol and OS value (R^2 =0.0003). Interestingly, it is observed that there exists a very weak negative linear relation when individual phenolic compounds 4-hydroxyphenyl acetic acid, vanillic acid, caffeic acid and p-coumaric acids and OS are compared and R^2 values of 0.46, 0.39, 0.37, 0.46 are obtained, respectively while luteolin shows a very weak positive linear relation with the value of R^2=0.31. It is expected that these individual compounds should contribute positively to the olive oil stability but it is not always the case in here. Synergistic action of individual phenolic compounds also needs to be taken into consideration as far as the antioxidant effect is concerned.

Table 4.7. Individual phenolic compounds (in mg/kg) of olive oils from Karaburun region

Phenolics	Eğlenhoca(EH)			Karareis(RS)			Karapınar(KP)		
	Mean	Range		Mean	Range		Mean	Range	
	(mg/kg)	Min	Max	(mg/kg)	Min	Max	(mg/kg)	Min	Max
Hxty[1]	4.44±1.95	1.98	7.25	2.24±1.96	0.85	3.63	1.43±0.76	0.98	2.30
Tyrs[2]	6.93±2.96	3.83	11.33	4.11±0.46	3.79	4.44	9.36±3.11	5.80	11.50
4-Hypa[3]	0.29±0.07	0.20	0.41	0.64±0.28	0.44	0.84	0.53±0.33	0.23	0.89
3-Hypa[4]	0.57±0.14	0.43	0.75	0.35±0.03	0.33	0.37	0.44±0.13	0.34	0.59
Vna[5]	0.43±0.11	0.31	0.58	0.79±0.13	0.69	0.88	0.57±0.28	0.25	0.75
Sya[6]	0.09±0.04	0.05	0.16	0.21±0.24	0.04	0.38	0.05±0.05	nd	0.09
Cina[7]	0.04±0.01	0.02	0.06	0.07±0.08	0.01	0.13	0.03±0.04	nd	0.07
O-cou[8]	nd	nd	0.01	0.08±0.10	0.01	0.15	nd	nd	nd
2-3 Dhxyb[9]	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chla[10]	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cfa[11]	0.03±0.03	0.01	0.10	0.06±0.03	0.04	0.08	0.04±0.06	nd	0.11
Vnl[12]	0.12±0.04	0.06	0.16	0.11±0.04	0.08	0.14	0.10±0.03	0.07	0.13
P-cou[13]	0.18±0.13	0.02	0.44	0.37±0.37	0.11	0.62	0.98±0.55	0.40	1.49
Apig[14]	0.10±0.25	nd	0.66	1.27±0.96	0.60	1.95	2.18±0.74	1.39	2.85
Lut[15]	0.84±0.66	0.12	1.80	0.04±0.01	0.03	0.05	0.18±0.19	nd	0.38

[1] hydroxytyrosol, [2] tyrosol, [3] 4-hydroxybenzoic acid, [4] 3-hydroxybenzoic acid, [5] vanillic acid, [6] syringic acid, [7] cinnamic acid, [8] o-coumaric acid, [9] 2-3 dihdroxybenzoic acid, [10] chlorogenic acid, [11] caffeic acid, [12] vanillin, [13] p-coumaric acid, [14] apigenin, [15] luteolin, nd: not determined

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Table 4.7.(cont.) Individual phenolic compounds (in mg/kg) of olive oils from Karaburun region

Phenolics	Barbaros(BR)			Gülbahçe(GB)			Torasan(TR)		
	Mean (mg/kg)	Range		Mean (mg/kg)	Range		Mean (mg/kg)	Range	
		Min	Max		Min	Max		Min	Max
Hxty	7.71±6.89	0.38	16.88	5.85±10.82	0.27	27.66	5.12±8.06	0.11	26.31
Tyrs	16.39±10.78	5.70	30.49	8.48±7.96	3.47	24.45	15.21±15.36	2.85	44.19
4-Hypa	0.86±0.57	0.20	1.37	0.48±0.22	0.24	0.75	1.34±1.69	0.19	5.99
3-Hypa	0.72±0.11	0.63	0.88	0.48±0.25	0.12	0.81	0.78±0.61	0.21	2.27
Vna	1.17±0.46	0.76	1.81	0.58±0.31	0.14	0.95	1.02±0.77	0.23	2.87
Sya	0.10±0.04	0.07	0.15	0.08±0.06	0.03	0.18	0.09±0.04	0.03	0.14
Cina	0.07±0.06	0.04	0.16	0.07±0.05	0.02	0.17	0.09±0.13	nd	0.41
O-cou	nd	nd	nd	nd	nd	nd	0.05±0.10	nd	0.30
2-3 Dhxyb	0.04±0.05	nd	0.10	nd	nd	nd	0.01±0.02	nd	0.05
Chla	0.02±0.03	nd	0.06	0.00±0.01	nd	0.02	0.01±0.02	nd	0.05
Cfa	0.09±0.02	0.06	0.10	0.07±0.03	0.02	0.11	0.12±0.13	0.02	0.45
Vnl	0.13±0.09	0.06	0.26	0.10±0.08	0.04	0.26	0.06±0.04	nd	0.12
P-cou	0.96±0.44	0.59	1.46	1.16±0.63	0.36	1.84	1.67±2.33	0.20	8.13
Apig	1.28±0.55	0.62	1.80	2.10±1.11	0.65	3.68	1.44±1.66	0.04	5.29
Lut	0.03±0.04	nd	0.09	0.15±0.18	nd	0.47	0.08±0.12	nd	0.38

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Table 4.7.(cont.) Individual phenolic compounds (in mg/kg) of olive oils from Karaburun region

Phenolics	Kuşcular(KS)			Özbek(OZ)			Urla(UR)		
	Mean (mg/kg)	Range		Mean (mg/kg)	Range		Mean (mg/kg)	Range	
		Min	Max		Min	Max		Min	Max
Hxty	4.65±5.20	0.91	13.77	5.96±8.38	0.48	30.72	3.91±3.84	0.66	9.95
Tyrs	10.66±7.54	4.52	23.68	11.60±7.68	3.58	31.18	8.64±13.01	1.22	31.74
4-Hypa	0.92±0.27	0.65	1.35	0.83±0.65	0.18	2.14	0.29±0.27	nd	0.64
3-Hypa	0.57±0.15	0.34	0.73	0.62±0.28	0.19	0.97	0.38±0.23	0.08	0.65
Vna	1.20±0.25	0.94	1.61	0.99±0.45	0.41	1.65	0.45±0.27	0.19	0.82
Sya	0.08±0.02	0.05	0.11	0.07±0.04	nd	0.16	0.07±0.04	0.02	0.14
Cina	0.12±0.12	nd	0.29	0.06±0.07	nd	0.23	0.03±0.04	nd	0.08
O-cou	0.01±0.02	nd	0.04	nd	nd	nd	nd	nd	nd
2-3 Dhxyb	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chla	0.01±0.02	nd	0.04	0.01±0.02	nd	0.05	nd	nd	nd
Cfa	0.26±0.21	0.03	0.60	0.17±0.10	0.04	0.35	0.03±0.02	0.01	0.05
Vnl	0.18±0.15	0.06	0.42	0.31±0.31	0.01	1.14	0.16±0.14	nd	0.33
P-cou	2.39±1.76	0.28	5.01	1.315±0.780	0.45	2.48	0.26±0.23	0.03	0.51
Apig	0.74±0.75	0.05	1.97	1.60±1.10	nd	3.35	0.53±0.47	0.06	1.12
Lut	0.59±0.71	0.02	1.59	0.17±0.30	nd	0.88	0.01±0.03	nd	0.06

(cont. on next page)

Table 4.7.(cont.) Individual phenolic compounds (in mg/kg) of olive oils from Karaburun region

Phenolics	Unknown (UK)		
	Mean (mg/kg)	Range	
		Min	Max
Hxty	5.57±5.40	0.09	18.74
Tyrs	11.96±11.73	0.73	38.65
4-Hypa	0.66±0.53	0.18	1.56
3-Hypa	0.70±0.59	0.15	1.88
Vna	0.69±0.38	0.22	1.31
Sya	0.06±0.04	0.01	0.16
Cina	0.04±0.05	nd	0.13
O-cou	0.05±0.10	nd	0.30
2-3 Dhxyb	nd	nd	nd
Chla	nd	nd	nd
Cfa	0.07±0.04	0.02	0.13
Vnl	0.13±0.18	nd	0.52
P-cou	0.76±0.90	0.07	2.53
Apig	0.57±0.72	nd	2.29
Lut	0.69±0.88	nd	2.55

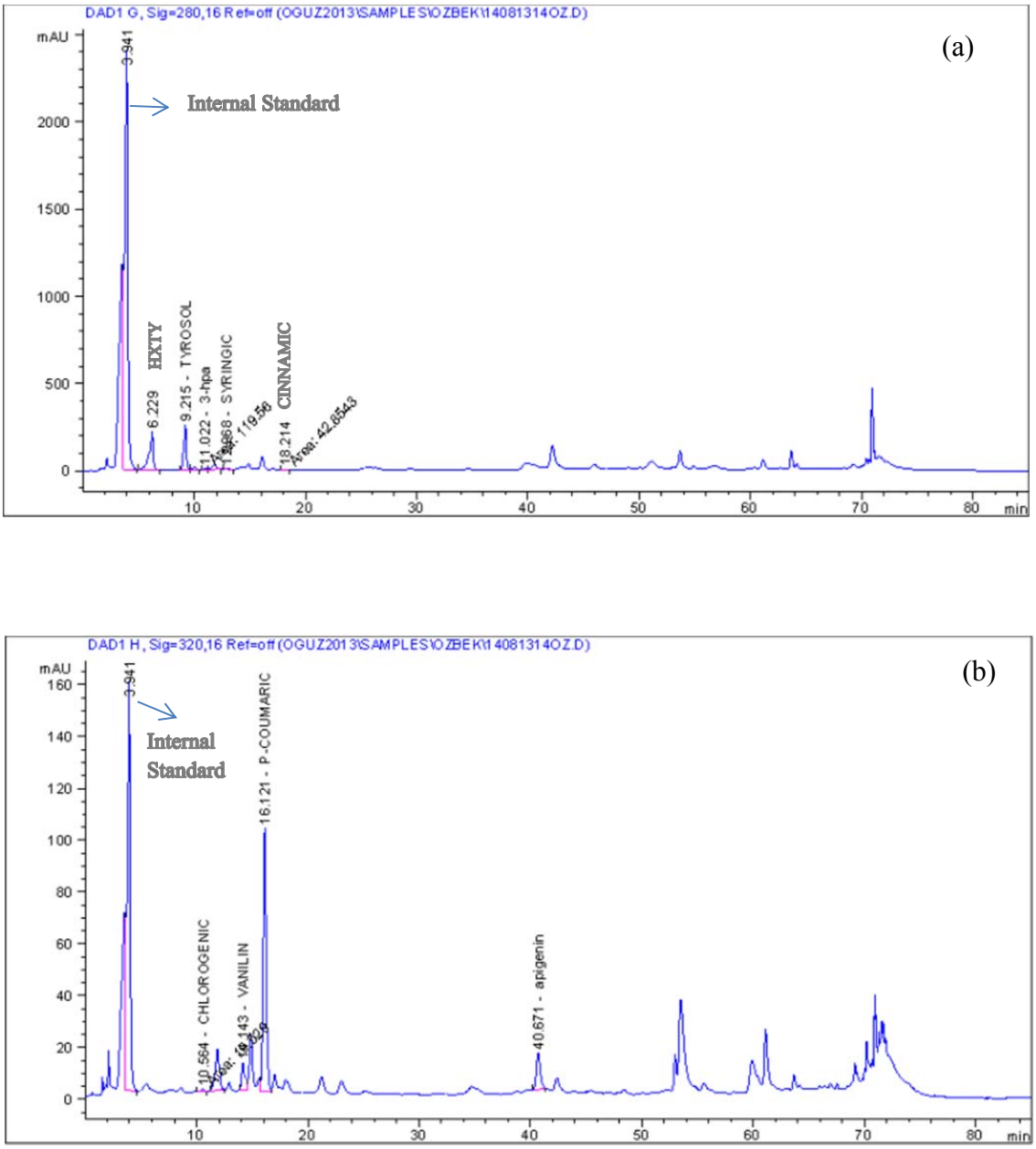


Figure 4.2. Typical HPLC chromatogram of the phenolic extract of olive oils from Karaburun region (a) at 280 nm (b) at 320 nm

4.1.5. FTIR Profile

According to the literature mid-infrared profile evaluated by Fourier transform infrared spectroscopy (FTIR) has been mainly used to detect adulteration as well as in classification purposes due to short analysis time, easy sample preparation, reproducibility and non-destructiveness of the technique (Christy et al., 2004; Galtier et al., 2008; Lai et al.1995; Yang et al., 2005). Working principle of the method is based on the characteristic vibrational and rotational modes (stretching, bending, wagging and rocking) of each specific molecular group at different wavelengths with absorption of infrared radiation (Vlachos, et al.2006). Mid-IR spectrum is in the range of 4000-400 cm^{-1}; however, 4000-650 cm^{-1} is the most commonly used region to eliminate the noise at the edges. In Figure 4.3, chemical groups which correspond to the defined peaks in a FTIR spectrum are shown. Peak at 2950-2800 cm^{-1} region is associated with C-H stretching vibrations while another peak around 1745 cm^{-1} results from C=O double bond stretching vibration. Fingerprint region corresponds to 1250 and 700 cm^{-1} area because of stretching vibrational movement and rocking vibration of C-O ester group and CH_2, respectively. 1470-1200 cm^{-1} range is known for CH bending (Harwood & Aparicio 2000). With the naked eye detection of differences in the spectral peaks of olive oils from different regions is impossible; therefore, all the FTIR data are evaluated with multivariate statistical analysis.

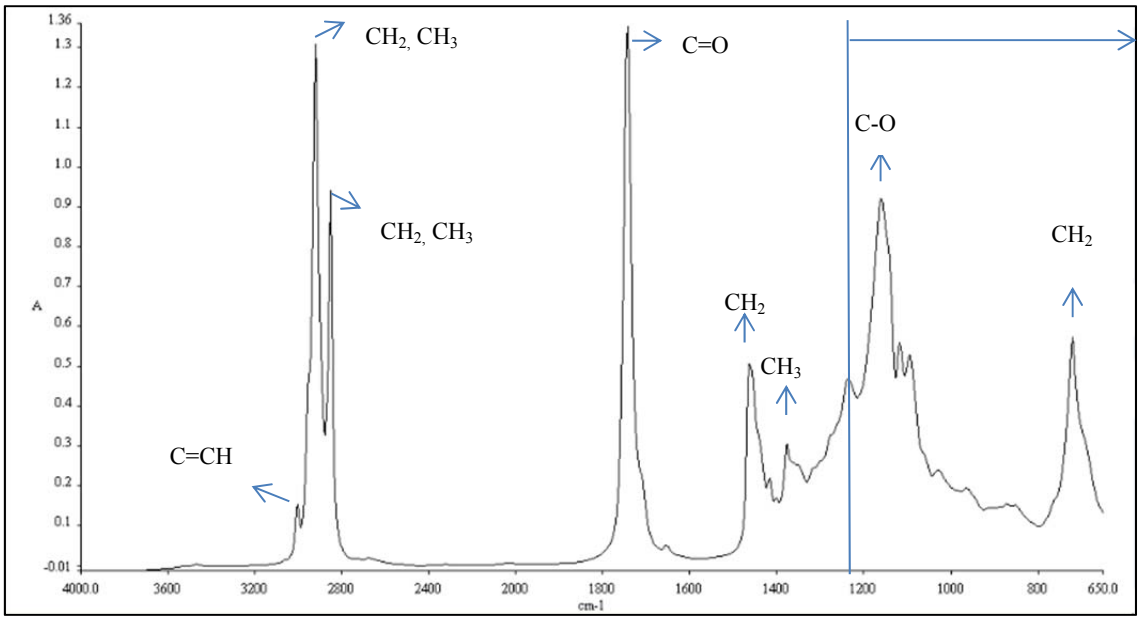


Figure 4.3. Typical FTIR spectrum of olive oils from Karaburun region
(Area shown with arrow indicates fingerprint region)

4.2. Classification of Olive Oils from Karaburun Region

4.2.1. Classification Using Fatty Acid Profile

In order to examine the geographical location effect on fatty acid profile a multivariate data set of 19 fatty acid variables of 54 observations were used. This data set was examined with PCA to observe the differences between locations. Figure 4.4 shows the score and loading plots of the two components PCA model with R^2= 0.326.

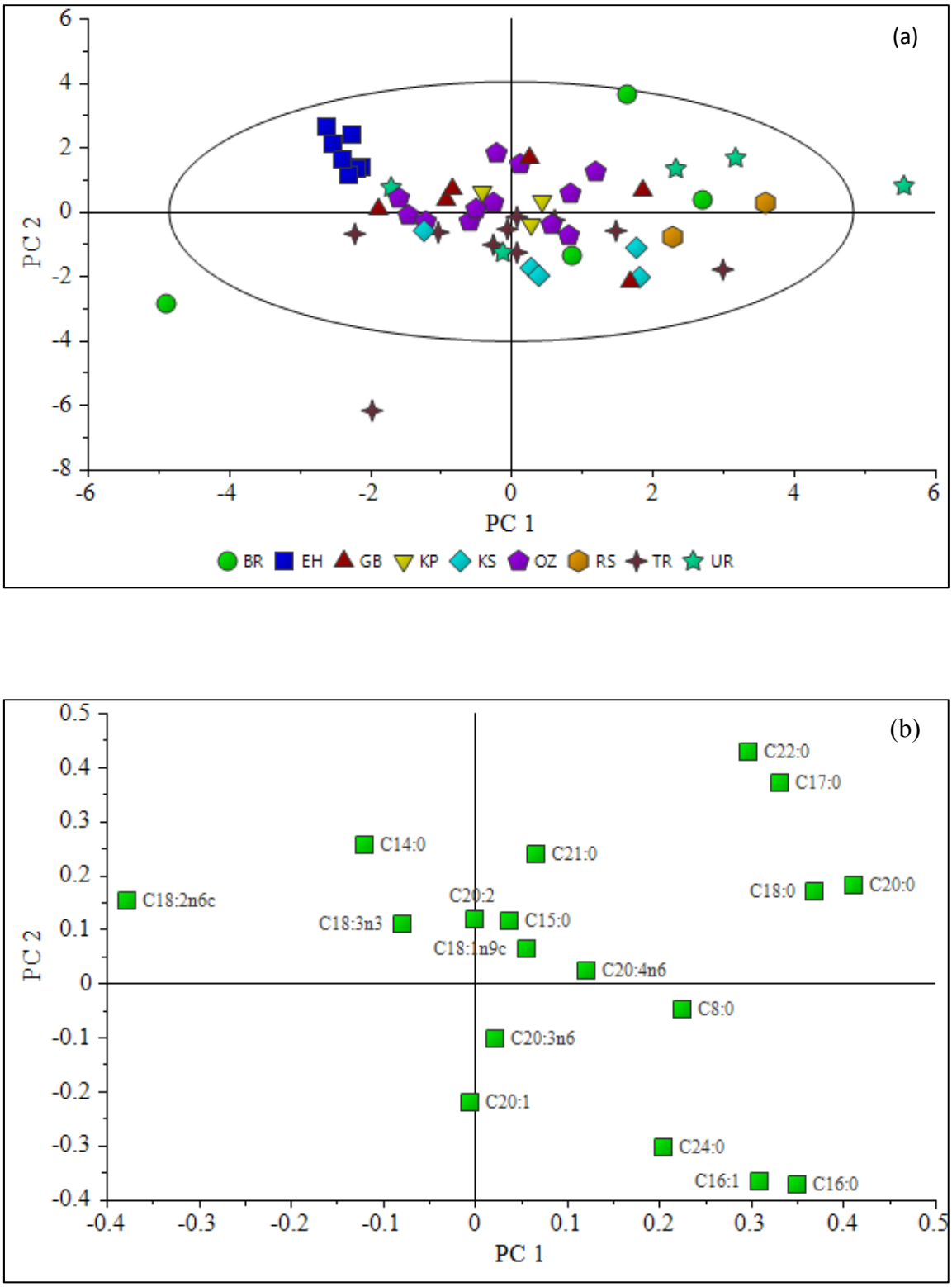


Figure 4.4. (a) Score plot & (b) loading plot of fatty acids of olive oils from different regions with PCA

From the score plot it could be clearly seen that EH region is quite different than the rest of the group. For the rest of the regions there seemed to be no clear differences between groups. Loading plot for this model is also presented in Figure 4.4 (b) and this

plot shows which fatty acids are responsible for differentiation. For this case, C18:2n6c and C18:3 are the most effective variables on the separation of EH region. In the literature, these two fatty acids as well as C16:0 were indicated as the fatty acids with high differentiation power (D'Imperio, et al. 2007). In the present study, even if the most of the parameters like olive variety, extraction method and harvest year were the same for each olive oil sample except their geographical locations (located in a narrow range, in some points very close to each other but eventually different) a slight differentiation is still observed.

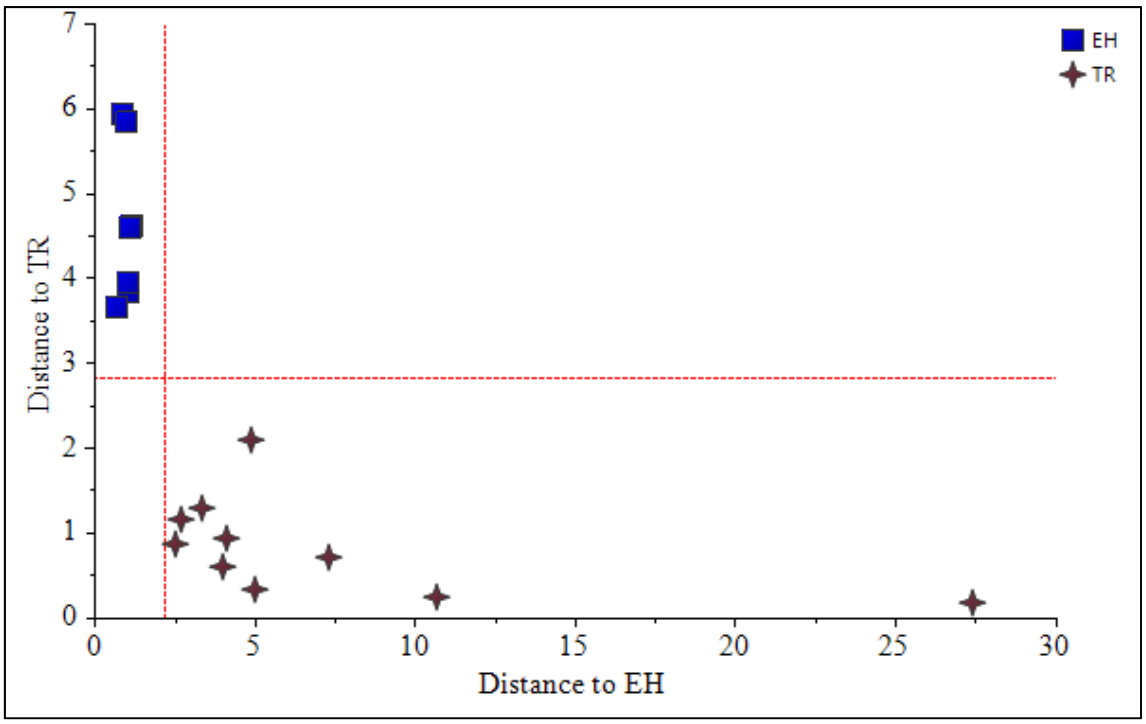


Figure 4.5. Coomans' plot for the classification with respect to EH and TR olive oils

Main idea of constructing a Cooman's plot is to see how well the principal groupings could be separated from each other. SIMCA results for the models developed with fatty acid profile are presented in Table 4.8. Model developed for EH with 2 PCs accounts for 76.7% of total variation (Table 4.8.). This model differentiates EH from the most of the other regions, and only one example plot is presented in Figure 4.5 which shows separation of EH and TR region's olive oil samples. According to this plot samples belonging to two different areas are located in their critical limits and there is no sample in the common region. Cooman's plots also show perfect discrimination between EH and KP, KS, OZ and RS regions with respect to their fatty acid profiles. In

the present study, there are 9 regions located in the Karaburun Peninsula but only one region, EH, was distinguished perfectly from the total of 5 regions, and the rest of 3 regions are not separated from each other very well (Figure 4.6). The reason could be that EH located closer to sea shore than the rest. A study in literature declared that the effect of cultivar is the most dominant factor in the olive oil classification according to fatty acid composition and it was also found out that geographical location also could have a minor effect on classification (D'Imperio 2007).

Table 4.8. SIMCA class models with respect to fatty acid profile

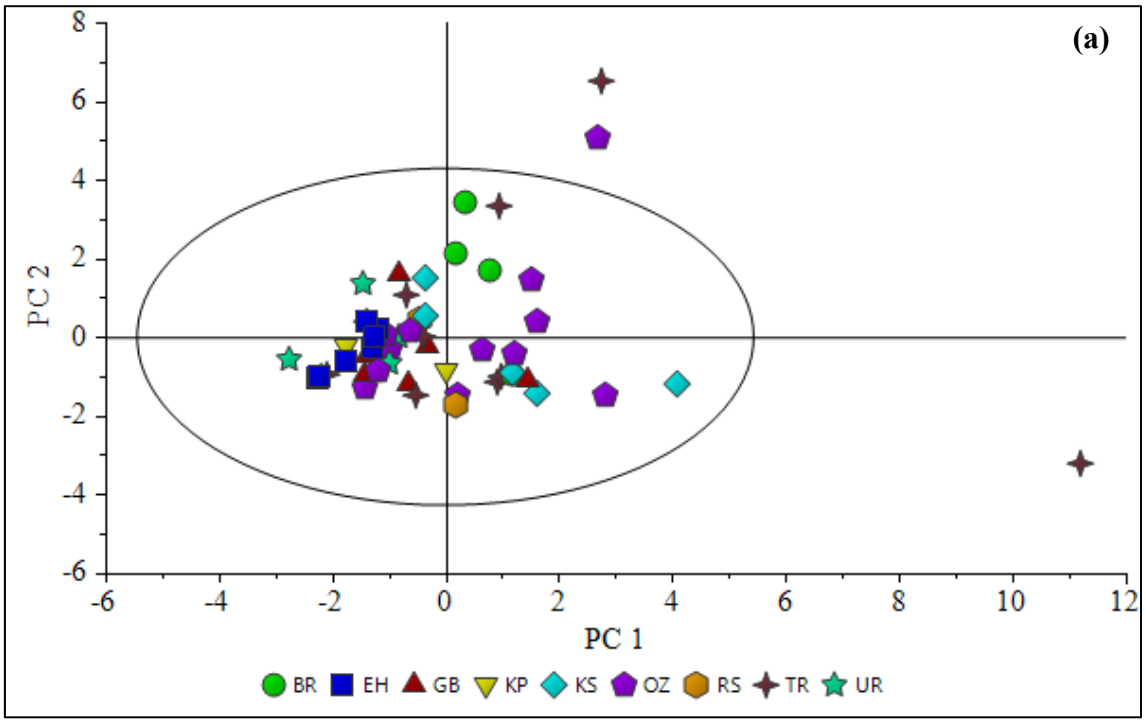
Location of olive oil	Number of PCs	R^2X (%)	Location of olive oil	Number of PCs	R^2X (%)
EH	2	76.7	TR	6	98.2
KP	1	73.5	KS	3	95.6
BR	2	89.2	OZ	7	96.9
GB	3	91.9	UR	3	97.2

(RS region is not shown due to low number of observation)

Figure 4.6. Classification map with respect to fatty acid profile (Source: Google Earth 2013) (dashed dialog box indicates only perfectly separated regions from the define area)

4.2.2. Classification Using Phenolic Profile

To examine the classification power of phenolic composition on olive oil, a multivariate set of 15 phenolic compounds of 54 observations were used. Two component PCA (R^2 = 0.49) score and loading plots are shown in Figure 4.7 (a) and 4.7 (b), respectively.



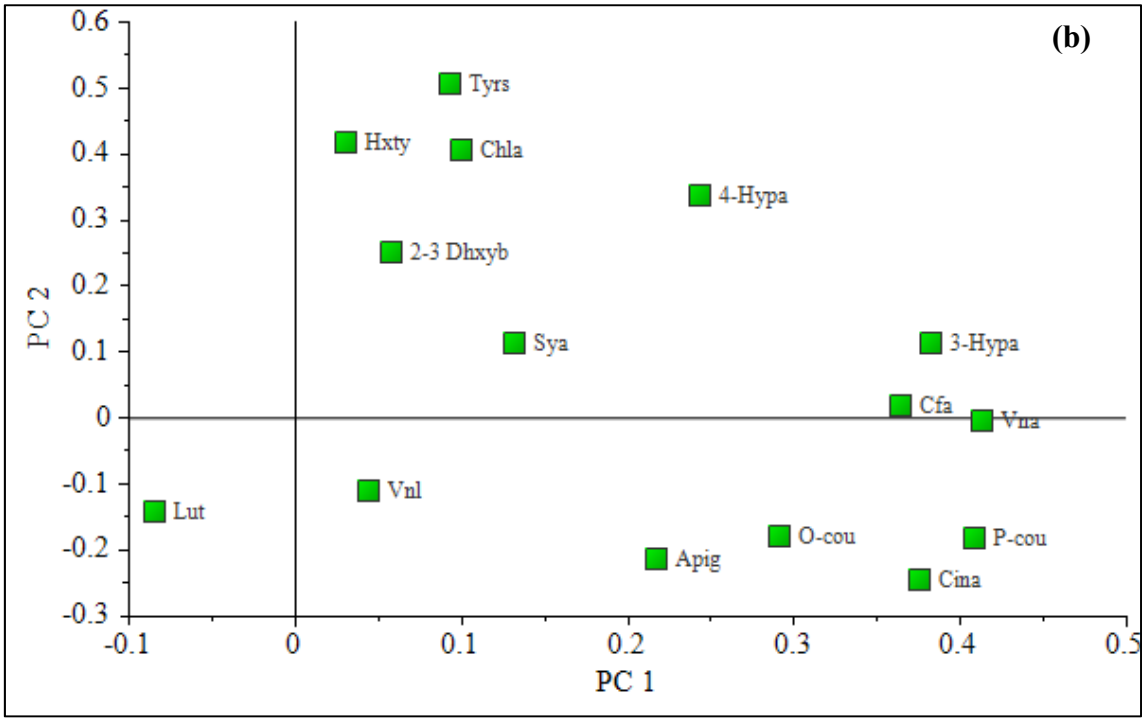


Figure 4.7. (a) Score plot & (b) loading plot of phenolic profiles of olive oils from different regions with PCA

According to the score plot good differentiation is observed for EH and BR regions. Loading plot revealed that high luteolin content differentiates EH from the rest

of the group while high amount of tyrosol, hydroxytyrosol and chlorogenic acid separate BR region.

SIMCA class models were constructed for each region given in Table 4.9 and Figure 4.8 indicates well differentiation between EH and BR region in which samples belonging to two different regions do not exceed their critical limits. In the light of the class model EH region is also separated from RS region perfectly whereas from OZ and TR region slightly. RS region is distinguished from EH and BR region. The last well separated area is BR region which is explained by 2 PCs accounting for 92.1% of total variation. To sum up EH region classification, BR and RS regions are clearly separated from each other while for the rest there is no clear separation at all.

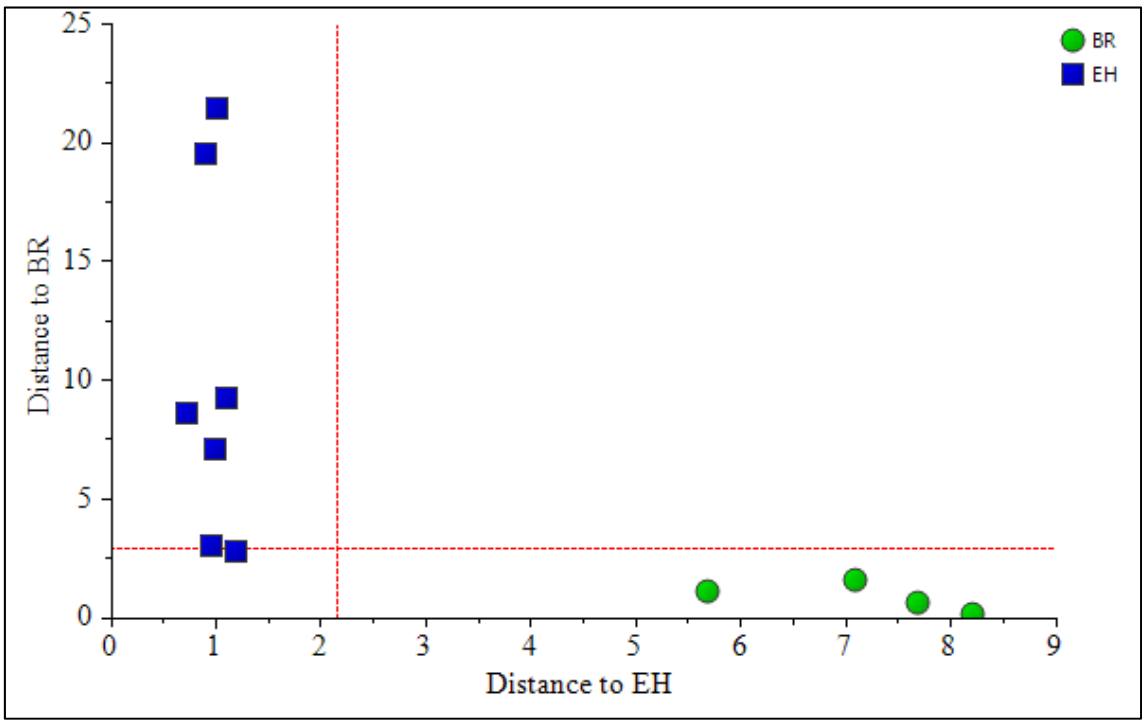


Figure 4.8. Coomans' plot for classification with respect to defined geographic locations (EH and BR) using phenolic composition

Table 4.9. SIMCA class models with respect to phenolic profile

Location of olive oil	Number of PCs	R^2X (%)	Location of olive oil	Number of PCs	R^2X (%)
EH	2	74.2	TR	6	98.6
KP	1	58.8	KS	2	75.8
BR	2	92.1	OZ	8	99.2
GB	2	80.8	UR	2	85.6

Geographical studies have been mostly focused on different cultivars in the literature but in the present study only one variety was investigated. In a study by Bakhouche et al. (2013), 18 phenolic compounds were identified to find out a relation between the amount of phenolic compounds and geographical origin for the same olive type, Arbequina, cultivated in different parts of southern Catalonia. Results of the study was quite promising, and it was determined that phenolic composition of olive oil is highly dependent on geographical area; therefore, phenolic composition could be used for classification studies even for the samples belonging to the same cultivar but grown in different areas (Bakhouche, et al. 2013). In the present study, only three different areas were differentiated from each other among nine groups and reason can be the close proximity of the regions (Figure 4.9). Only criterion in this study to create a group is the geographical location, because there was no chance to make detailed observations about the physical characteristics (solid type, irrigation regime etc.) of each area.

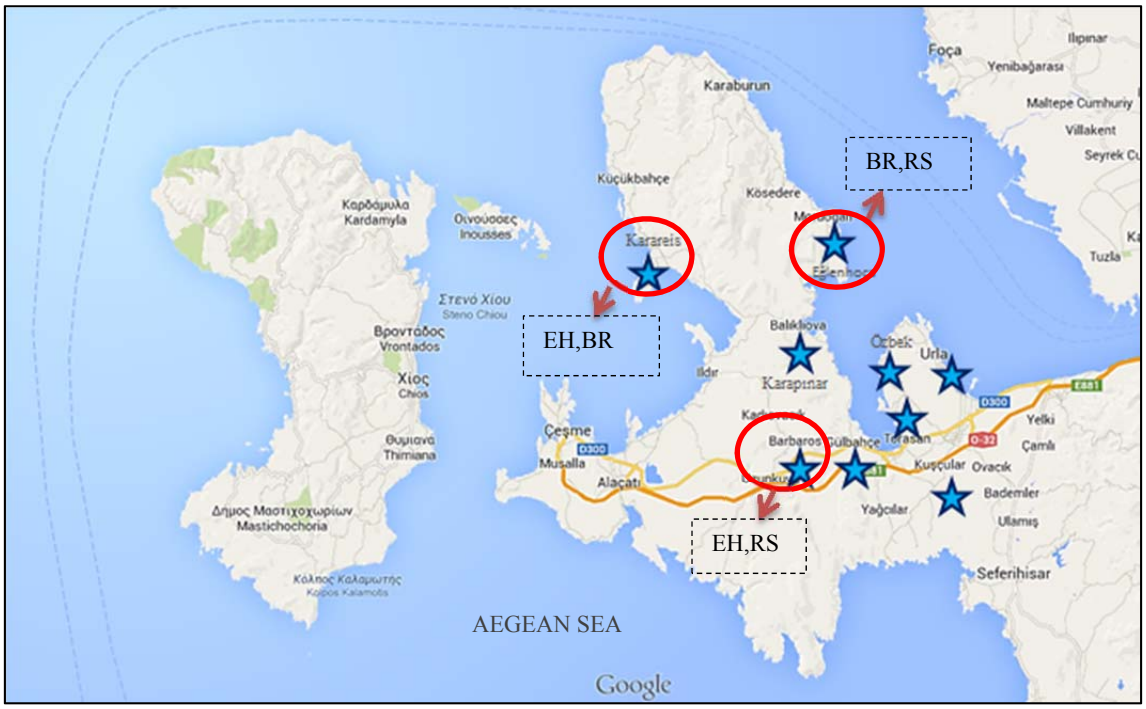


Figure 4.9. Classification map with respect to phenolic profile
(Source: Google Earth 2013)

When cumulative of TPC and phenolic compounds are considered together a new 2 PCs model explaining %49.4 of differences. However, SIMCA models were not as good as the previous one, only EH region could be differentiated from RS region perfectly also slight classification observed from BR, OZ and TR regions. SIMCA model parameters are given below in Table 4.10.

Table 4.10. SIMCA class models with respect to phenolic profile and TPC

Location of olive oil	Number of PCs	R^2X (%)	Location of olive oil	Number of PCs	R^2X (%)
EH	2	73.8	TR	6	98.6
KP	1	57.1	KS	2	76
BR	2	88.8	OZ	8	98.8
GB	2	80.1	UR	2	85.4

4.2.3. Classification Using FTIR Profile

FTIR data was used to investigate the classification of olive oils from Karaburun region. For this purpose, data was inserted into PCA to establish a score plot. 54

observations were evaluated with 4 PCs with R^2= 0.94. Score plot is shown in Figure 4.10. PCA was constructed by the first and the second PCs explaining 67.6% and 16.7% of total variation, respectively.

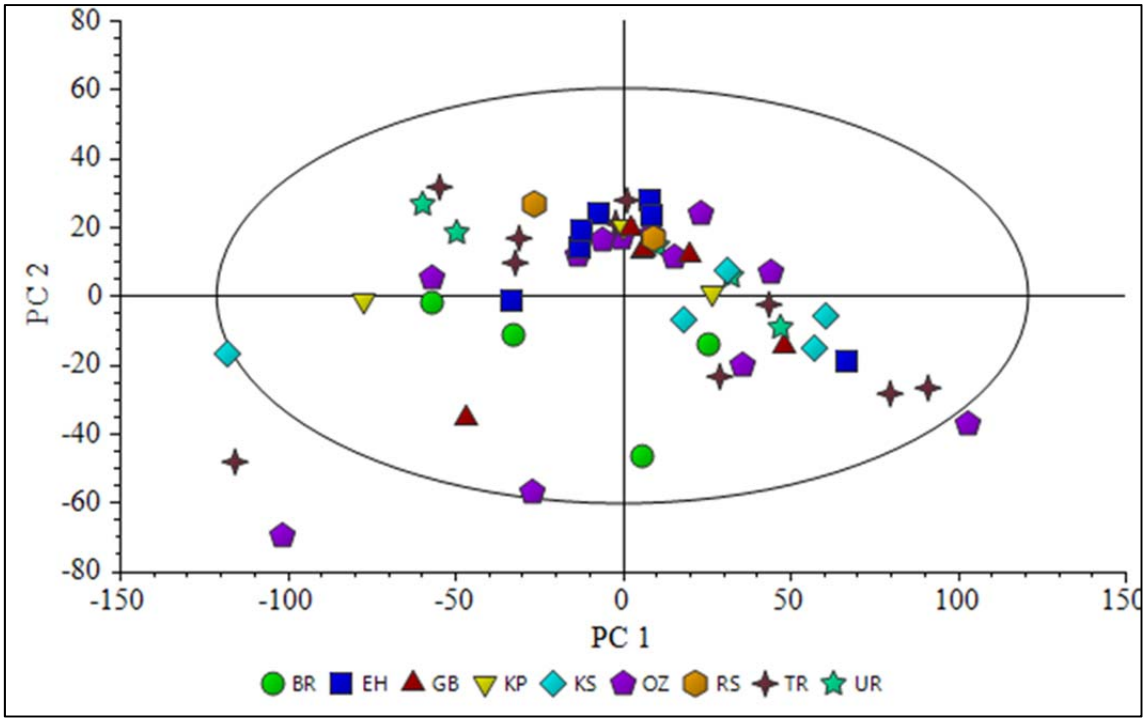


Figure 4.10. Score plot of FTIR profiles of olive oils from different regions with PCA

According to PCA score plot EH, BR and KS regions are grouped separately from each other, and clear separation is not observed for the rest of regions. In order to investigate differentiated groups more deeply, Cooman's plot is constructed (Figure 4.11). 9 class models are built and general statistics of these distinct classes are given in Table 4.11. EH region plotted against KS region is presented in Figure 4.11 as an example of perfectly differentiated regions. EH is also separated from BR, RS and UR regions clearly. RS region is separated from all the regions except OZ region. BR region is differentiated from UR, EH and RS regions while TR and KP regions are only separated from RS. KS and UR regions are distinguished out from BR and RS regions; moreover, UR region is also separated from EH region. OZ and GB regions are not separated from any regions. Separation map is provided in Figure 4.12.

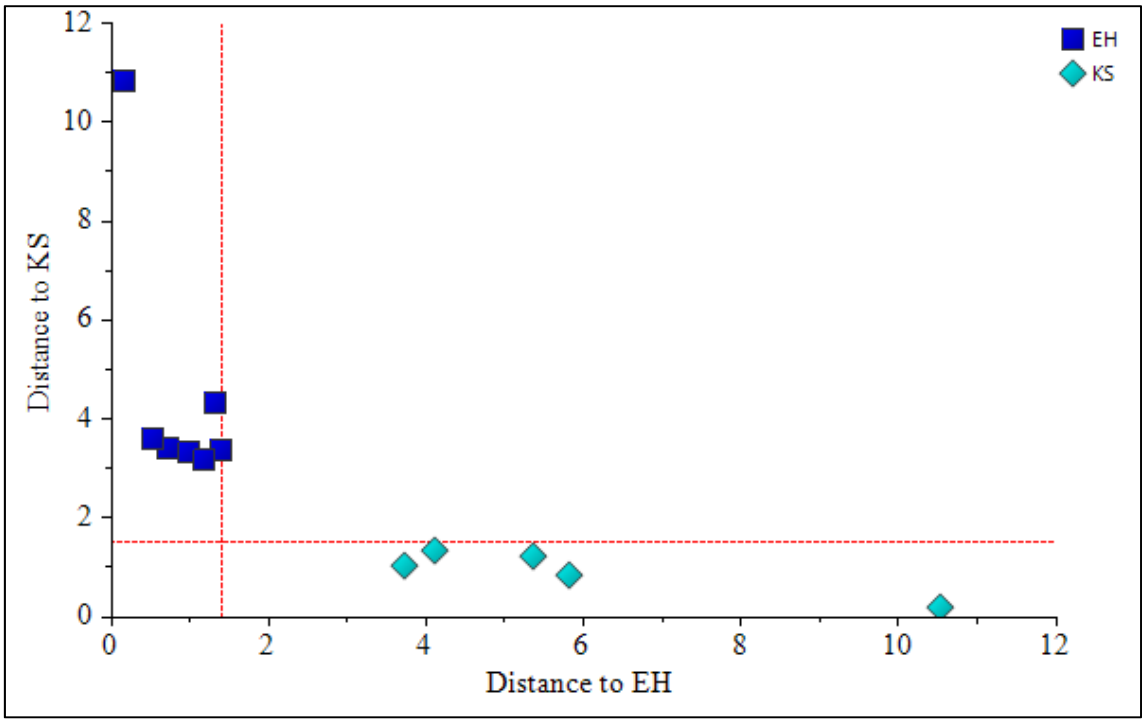


Figure 4.11. Coomans' plot for classification with respect to defined geographic locations (EH and KS) using FTIR profile

Table 4.11. SIMCA class models with respect to FTIR profile

Location of olive oil	Number of PCs	R^2X (%)	Location of olive oil	Number of PCs	R^2X (%)
EH	2	92.1	TR	6	99.6
KP	1	88	KS	2	97.8
BR	2	95	OZ	8	99.8
GB	2	87.8	UR	3	99.1

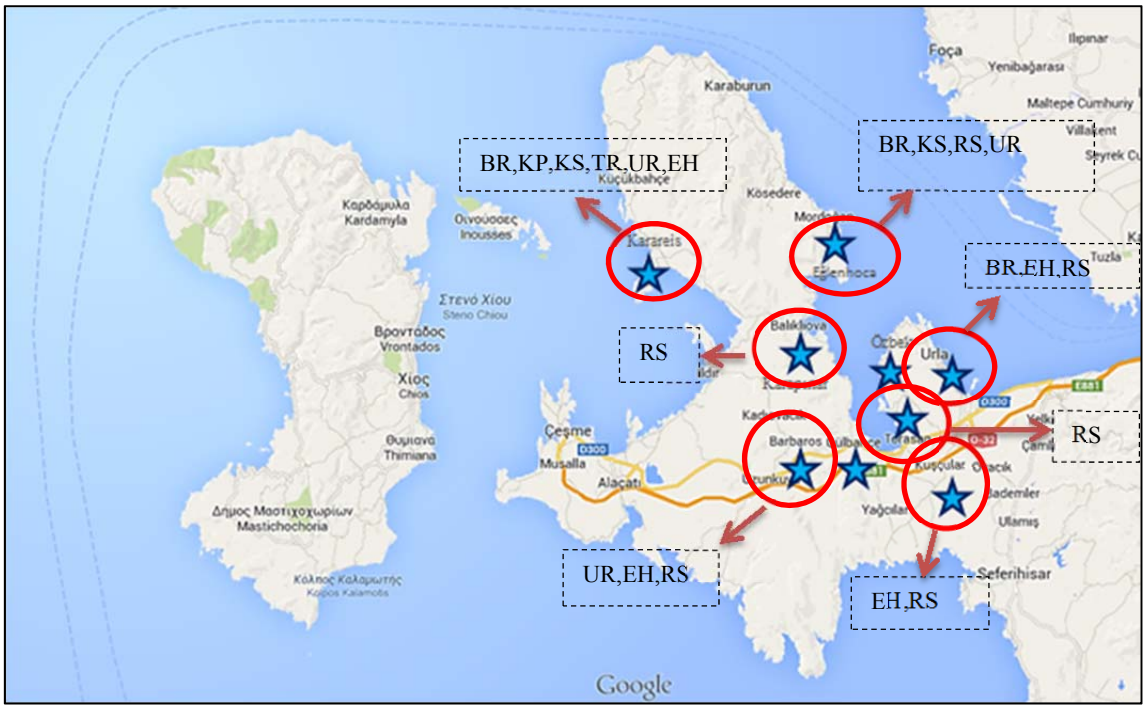


Figure 4.12. Classification map with respect to FTIR profile
(Source: Google Earth 2013)

Second derivative of the infrared data cluster is commonly used in data analysis to eliminate the shifts in the baseline and to reduce the edge effects PCA model constructed with transformed data and 6 PCs explain %39.5 of total variation. Score plot of the first two PCs obtained by PCA applied to the second derivative of FTIR profile of the olive oil is given below in Figure 4.13 which is able to reveal %23.9 of total variation.

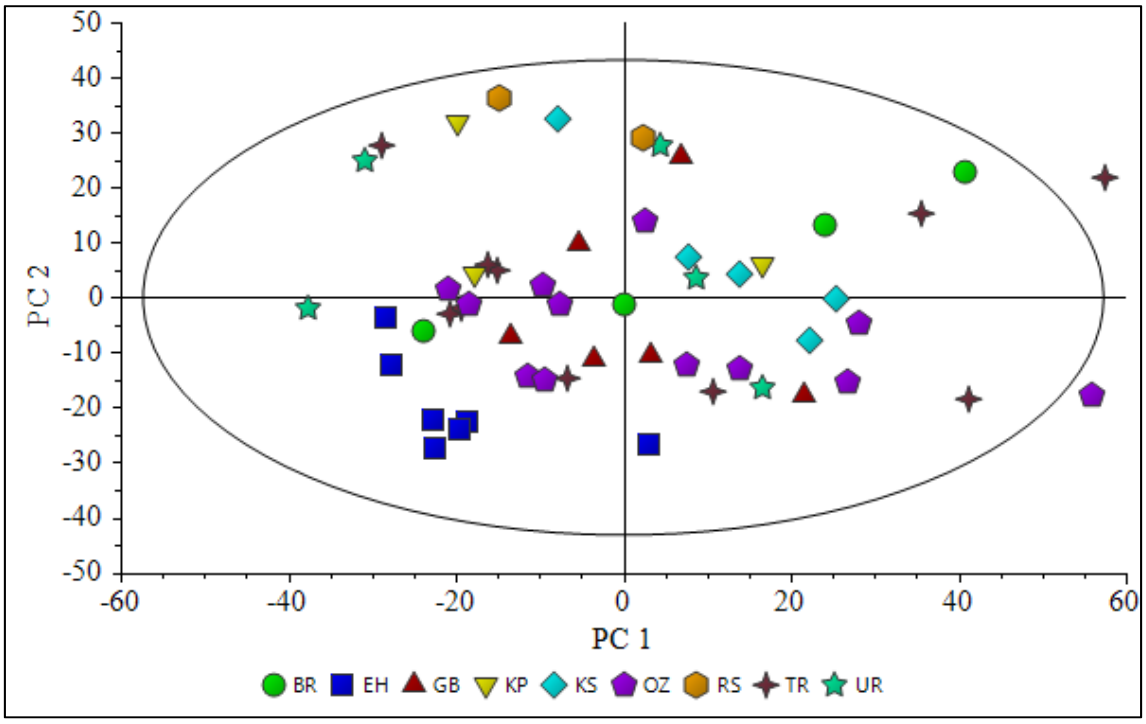


Figure 4.13. Score plot of PCs from second derivative of Karaburun Peninsula olive oils' FTIR spectra

From the figure above EH, KS and RS region oils clustered in different quarter of control ellipse. To visualize different classes more clearly SIMCA models are created for each defined region (Table 4.12). According to the Cooman's plots of the models, one of the best classified model, EH region plotted against GB region, is given in Figure 4.14. As it is seen in the figure samples do not exceed their critical limits and are perfectly classified.

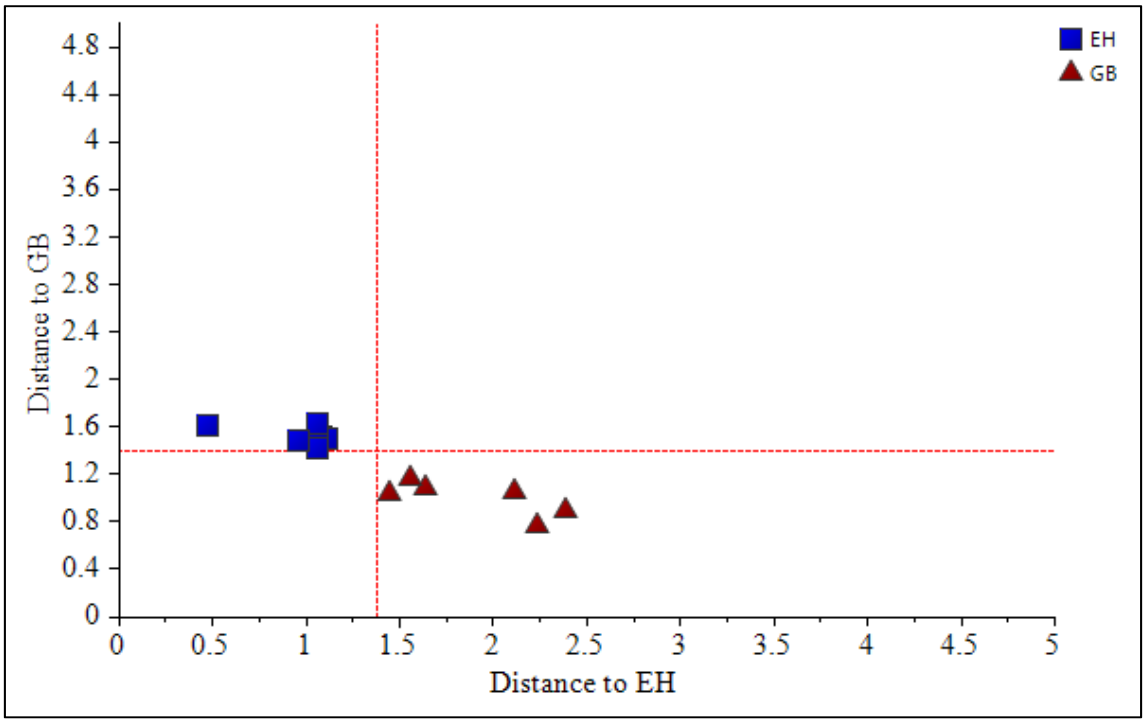


Figure 4.14. Coomans' plot for classification with respect to defined geographic locations (EH and GB) using second derivative of FTIR profile

Table 4.12. SIMCA class models with respect to second derivative of FTIR profile

Location of olive oil	Number of PCs	R^2X (%)	Location of olive oil	Number of PCs	R^2X (%)
EH	2	27.1	TR	6	41.7
KP	1	57.6	KS	2	41
BR	2	45.6	OZ	8	23.3
GB	2	28	UR	3	33

In general, EH region is successfully separated from all regions except TR and OZ regions. Actually TR region is not separated from any region at all. RS region is slightly differentiated from GB and UR regions while it is perfectly distinguished from EH, KP, BR, KS and OZ regions. KP and BR regions are separated well from each other, and both of them are also separated from the rest but OZ is poorly separated from GB just for BR region. GB and UR regions are separated from the same regions successfully but not from each other. OZ is only separated successfully from RS region and minor separation is observed from BR and KP regions. KS region is separated from

five different regions (UR, EH, RS, KP, BR). A summary of separation of regions according to their FTIR profile is shown in Figure 4.15.

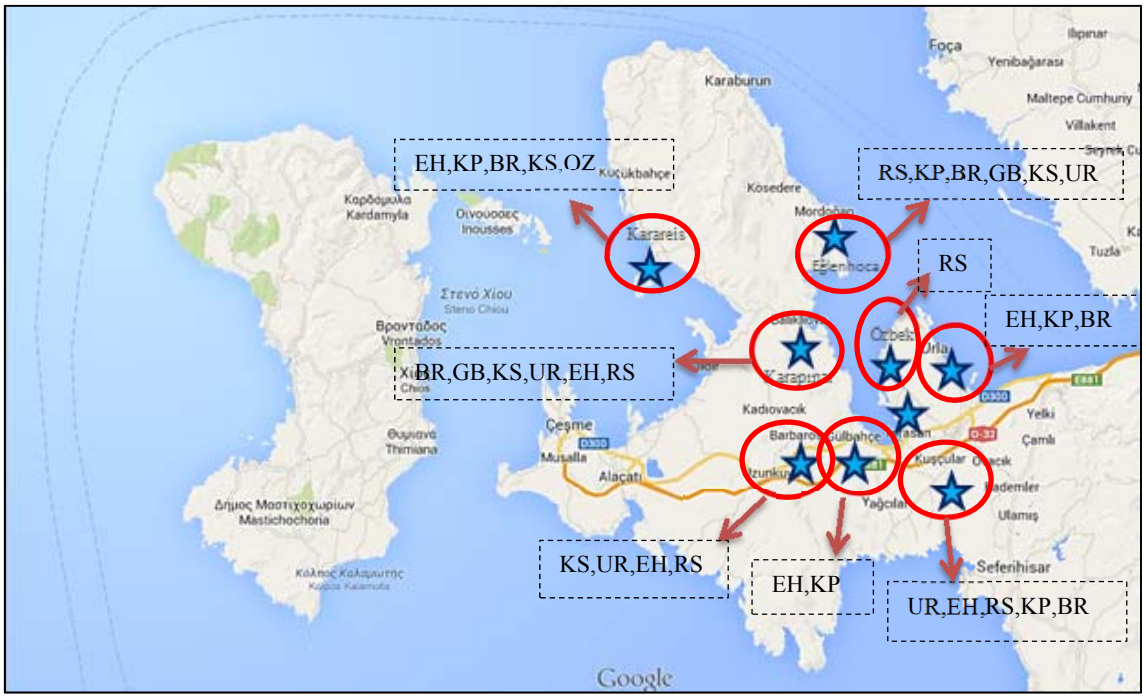


Figure 4.15. Classification map with respect to second derivative of FTIR profile (Source: Google Earth 2013)

PCA of the second derivative of the FTIR profile discriminates more area than the original spectra despite its low total variance. With the second derivative data 8 of the binary areas can be differentiated up to a certain extend while by original spectra 7 of the binary areas can be differentiated with a lower discriminatory power.

In the past studies, FTIR profile was used directly or with applying transformation. According to Galtier et. al (2008) six geographically close registered designation of origin regions (RDOs) could be satisfactorily classified by predicting fatty acids and triacylglycerols from FTIR spectra. In the study of Gurdeniz et al. (2008), in order to examine the variety effect in the classification, comparison of two varieties, Ayvalık and Gemlik, from two different areas of İzmir were used by two different data set of fatty acid profile and FTIR profile and it was observed that separation was successful to some extent by FTIR whereas differentiation by fatty acid profile was better compared to FTIR. In other studies usage of FTIR profile give promising results in classification manner by different research groups (Bendini, et al. 2007; Tapp, et al. 2003). In the present study, FTIR can differentiate most of the regions

successfully while only one region, EH, could be separated successfully with fatty acid profile. The reason could be that fatty acid profile based classification methods depend on mostly cultivar differences and minor effect of geographical location was observed as discussed before in the section 4.2.1. In the present study, olive samples were collected from a very narrow area; therefore, differences observed in fatty acid content of olive oils were not that significant. In the present study, the FTIR profile reveals more differentiation than the fatty acid content.

4.2.4. Classification Using Several Chemical Parameters

In this part, combinations of phenolic profile, TPC, OS, fatty acid profile, chlorophyll and carotenoid contents are used to classify olive oil samples. FTIR data were consciously omitted out from the data cluster since it has better differentiating power compared to other parameters; therefore, it dominates over the rest of chemical variables. PCA score plot of the data cluster constructed using these variables are given in the Figure 4.16.

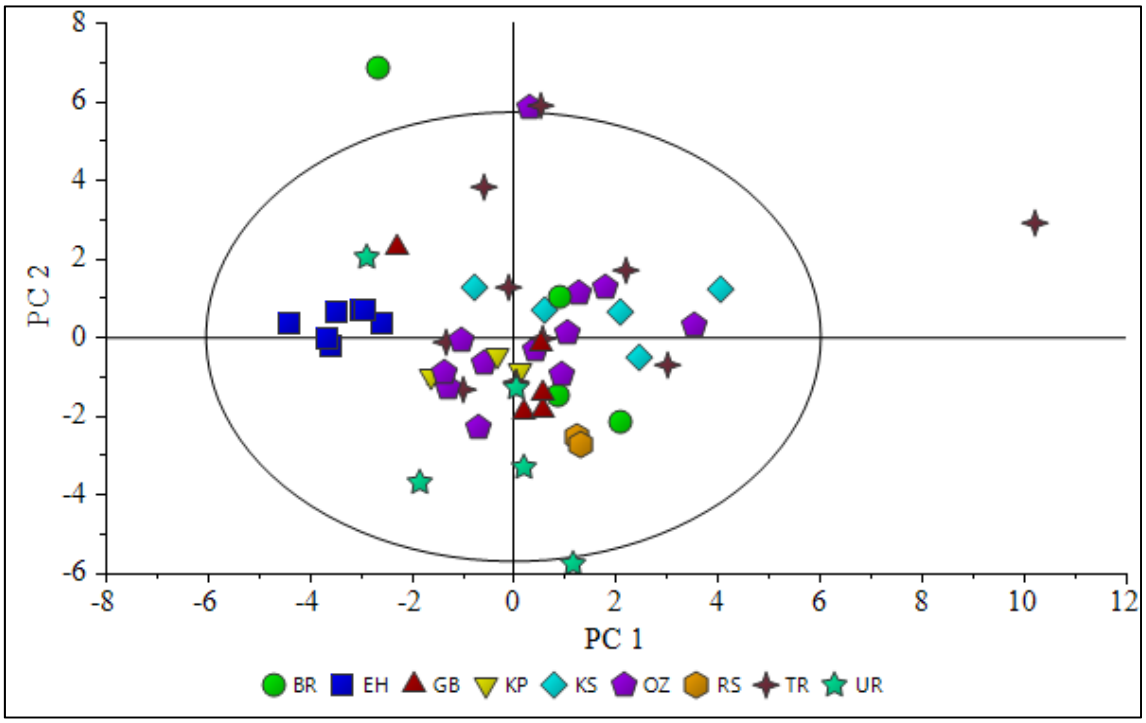


Figure 4.16. Score plot of all data (except FTIR) for the differentiation of olive oils from Karaburun region

PCA model is constructed using 37 variables with 54 observations giving a model with 2 PCs where first and second PCs explaining, %15.1 and %13.6 of total variation, respectively. Figure 4.17 shows the loading plot of the first two PCs. According to loading plot EH is differentiated from the rest by OS value, luteolin, linoleic acid and myrstic acid content.

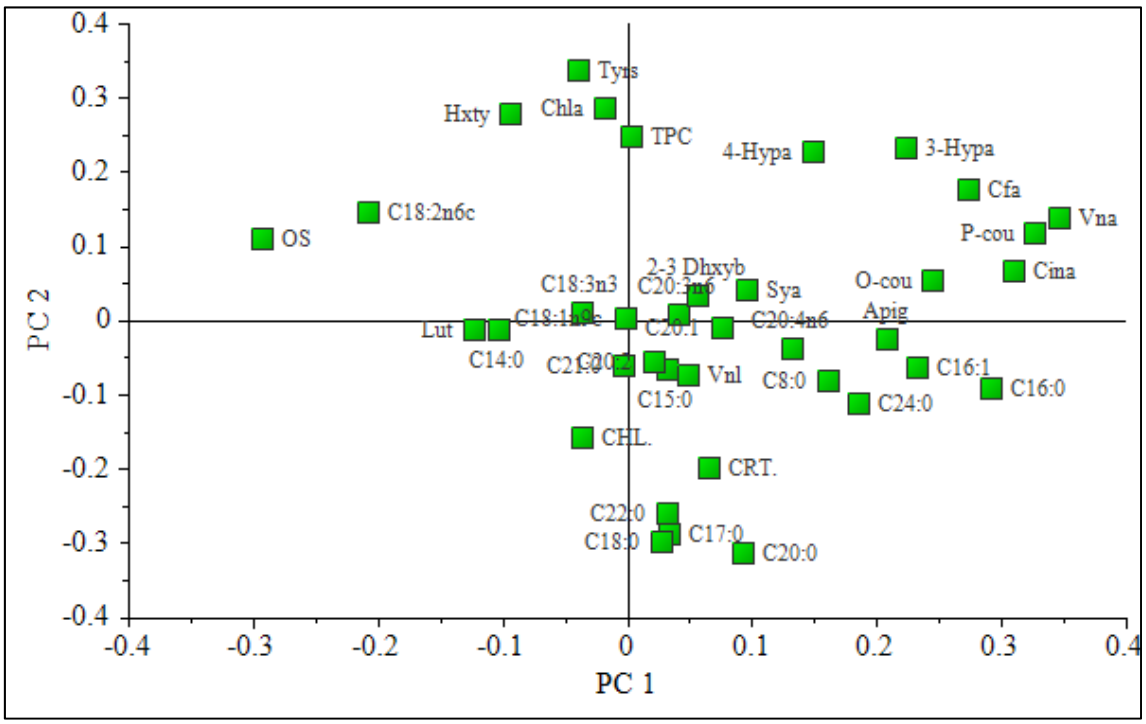


Figure 4.17. Loading plot of all data (except FTIR) for the differentiation of olive oils from Karaburun region

Cooman's plots are constructed for classification of regions with respect to various chemical data. 9 PCA classes were plotted against each other and in here only one of them (EH vs KP) is shown in Figure 4.18. The detailed information about the each PCA class model is provided in Table 4.13 except RS region due to low number of samples from this region. EH is successfully separated from KP, KS, RS and TR regions while it is slightly distinguished from BR and OZ regions. RS is differentiated from KP, KS, OZ, EH regions whereas weak separation is observed for TR. KP is differentiated only from EH and RS regions. OZ and GB regions are distinguished from the regional area RS and EH, respectively while KS region is separated from both RS and EH regions but they are not separated from each other. Differentiation of regions using various chemical parameters is shown on the map of Karaburun Peninsula (Figure 4.19).

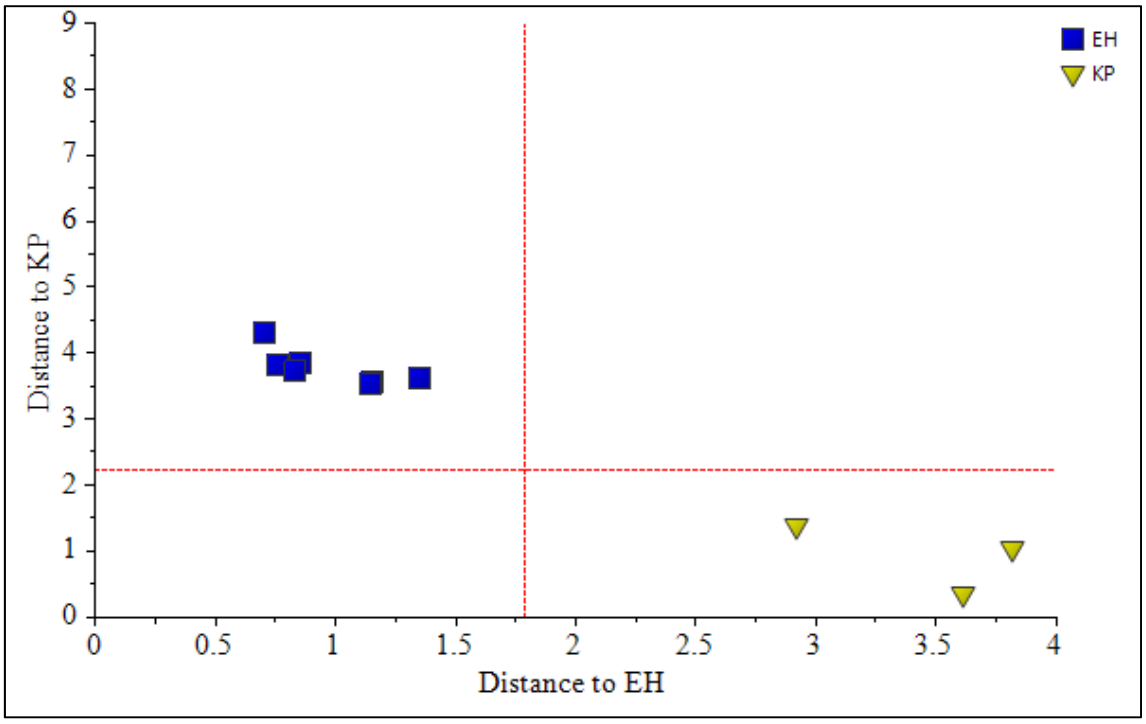


Figure 4.18. Coomans' plot for classification with respect to defined geographic locations (EH and KP) using all data (except FTIR)

Table 4.13. SIMCA class models with respect to all data (except FTIR)

Location of olive oil	Number of PCs	R^2X (%)	Location of olive oil	Number of PCs	R^2X (%)
EH	2	57.4	TR	7	97.2
KP	1	62.3	KS	1	38.9
BR	2	53.8	OZ	9	96.8
GB	2	67.4	UR	2	77.2

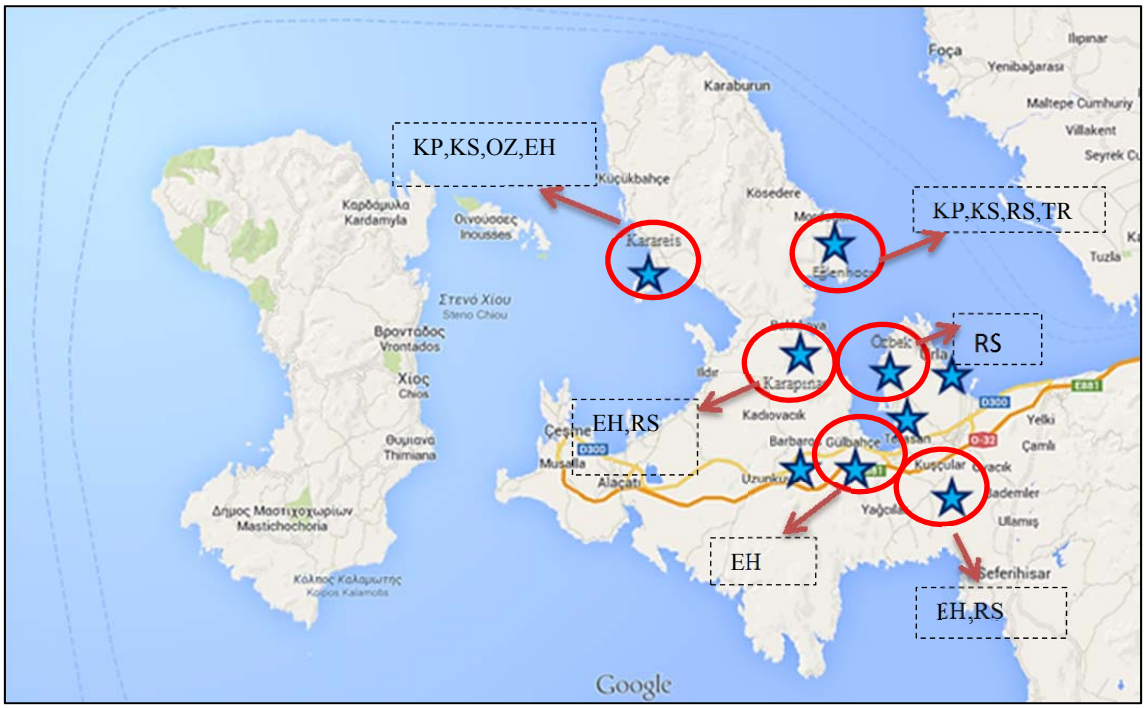


Figure 4.19. Classification map with respect to various chemical parameters for olive oils from Karaburun region (Source: Google Earth 2013)

4.2.5. Classification Using All Data

In this section, all chemical parameters as FTIR profile, phenolic compounds (including TPC), fatty acid profile, chlorophyll and carotenoid content are used in order to create 5 components PCA model with R^2= 0.952. First and second PCs explain %66.8 and %16.7 of total variation, respectively. According to score plot of the first two PCs given in Figure 4.20, EH samples seem to be grouped together while there is no clustering for the rest of the regions. SIMCA class models (Table 4.14) are also constructed for each region.

One of Coomans' plot is given in Figure 4.21 which indicates clear separation between EH and UR. General observations for different binary groups are as following: EH is further separated from BR, KS and RS perfectly while a slight differentiation from TR region also is observed. RS region is successfully separated from BR, KS, TR, EH and KP regions. BR region is distinguished from KS, UR and EH clearly while from TR, KP and RS regions slightly. Both UR and KS regions are separated from BR and EH regions whereas there was no differentiation between each other. Moreover, KS region is also distinguished from UR, KP and RS regions not perfectly and UR region is further separated from KS region marginally. KP region is only separated from RS

region perfectly while from BR and KS region slightly. TR region is imperfectly separated from just two regions BR, EH while clear separation was observed from RS region.

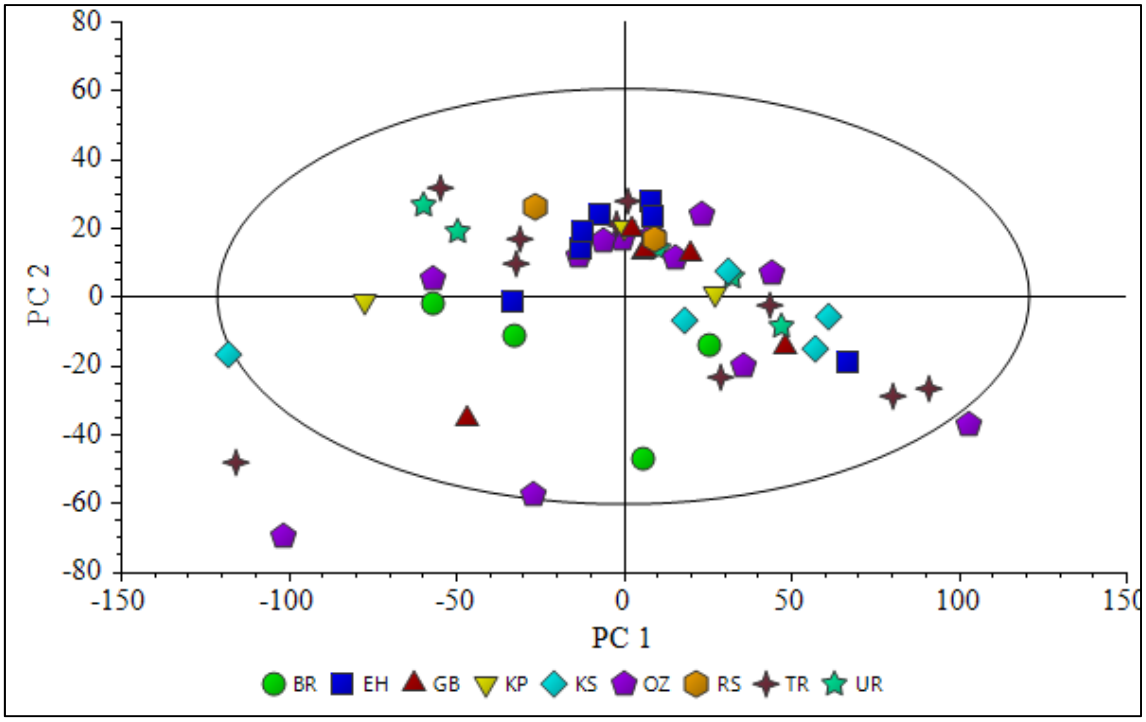


Figure 4.20. Classification map with respect to all chemical parameters for olive oils from Karaburun region

Table 4.14. SIMCA class models with respect to all chemical data

Location of olive oil	Number of PCs	R^2X (%)	Location of olive oil	Number of PCs	R^2X (%)
EH	2	91.7	TR	4	98.4
KP	1	87.8	KS	2	97.4
BR	2	94.8	OZ	2	90.7
GB	2	87.5	UR	3	99

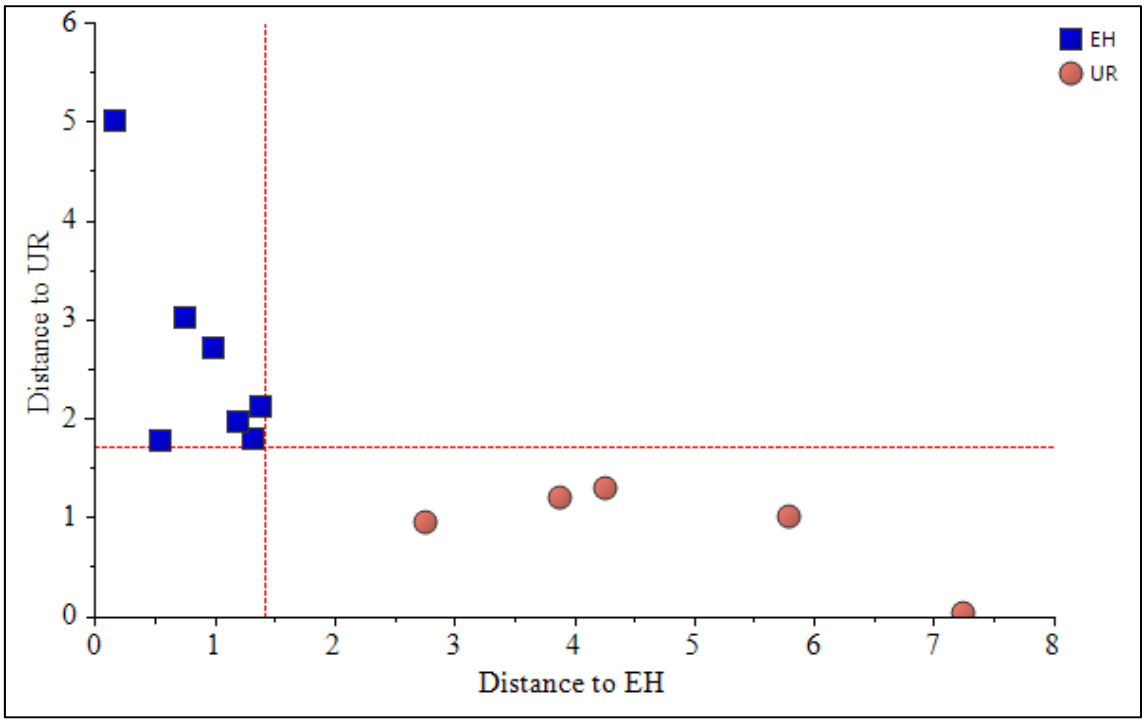


Figure 4.21. Coomans' plot for classification with respect to defined geographic locations (EH and UR) using all chemical data

General view of the Karaburun Peninsula map in terms of separation of olive oils from various locations is given below in Figure 4.22 according to all chemical data.

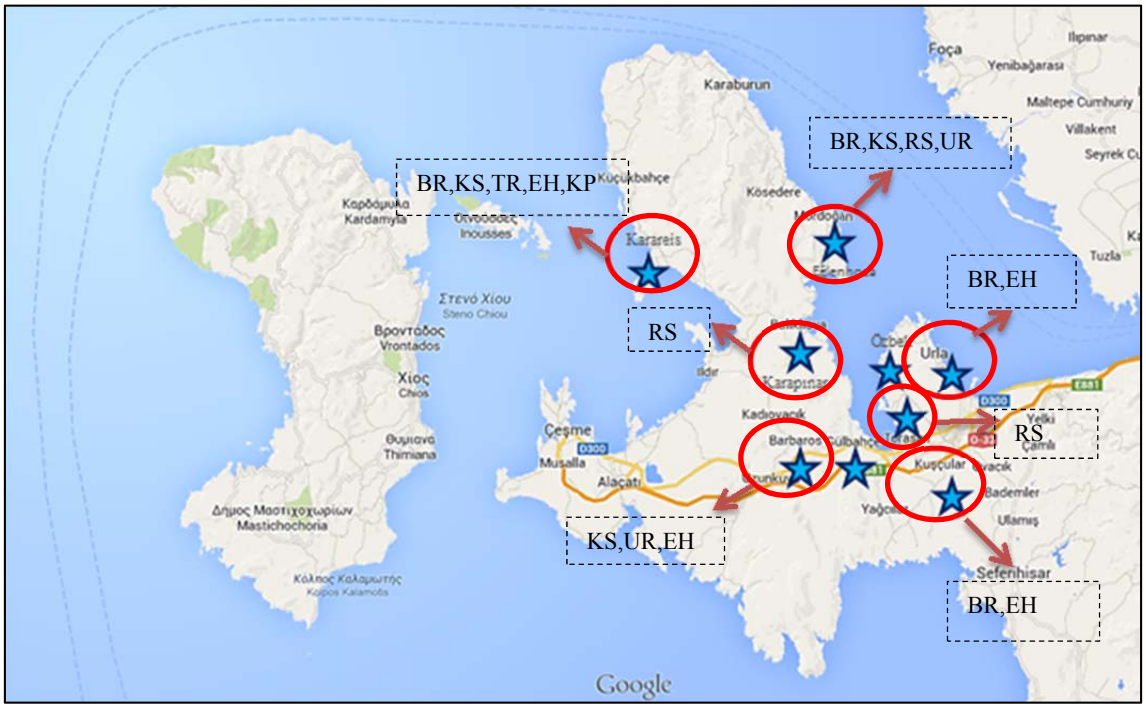


Figure 4.22. Classification map with respect to all chemical parameters (Source: Google Earth 2013)

4.3. Relationship Between FTIR Profile with Various Chemical Parameters and Various Chemical Parameters' Relation with Oxidative Stability

In the literature, FTIR spectra have been mainly used in classification purposes. Moreover, it has also gained popularity on quantitative analysis due to the fact that emitted IR energy is directly proportional to the amount of each individual compound concentration existed in the substance (Ismail et al., 1997; Ozturk et al., 2012). In this section, various chemical parameters such as oxidative stability, chlorophyll and carotenoid content, fatty acid profile (MUFA, PUFA, SFA included) and phenolic composition (TPC included) are tried to be estimated from FTIR spectra. Furthermore, OS is not only predicted from FTIR profile but also from combination of parameters like chlorophyll and carotenoid content, fatty acid profile and phenolic composition.

These parameters mentioned above are important quality parameters for olive oil. Therefore, it is important to determine these parameters in a fast and a reliable way. For this purpose, spectroscopic methods like nuclear magnetic resonance (NMR), near infra-red (NIR), mid-infrared (MIR) and Raman have been used and they are generally superior over time-consuming and expensive traditional chromatographic methods (Aparicio et al., 2013).

PLS regression was used to relate spectral data with analytical results of chemical parameters. Models were constructed for each response separately only with the exception of chemically similar constituents like phenolic compounds with TPC and fatty acids with PUFA, MUFA and SFA which were used in a single model. To evaluate the model performance cross-validation (leave one out) technique was used. To increase the efficiency of prediction one of the spectral filtering techniques, second derivation, was used whereas unmodified data of chlorophyll and carotenoid content, fatty acid profile and phenolic composition was used in oxidative stability prediction.

4.3.1. Prediction of Oxidative Stability From FTIR Profile

OS values are predicted from FTIR spectral data using PLS (Figure 4.23). The PLS model contains 5 PCs explaining 99% of the total variation of OS. Statistical parameters for calibration and cross validation are given in Table 4.15.

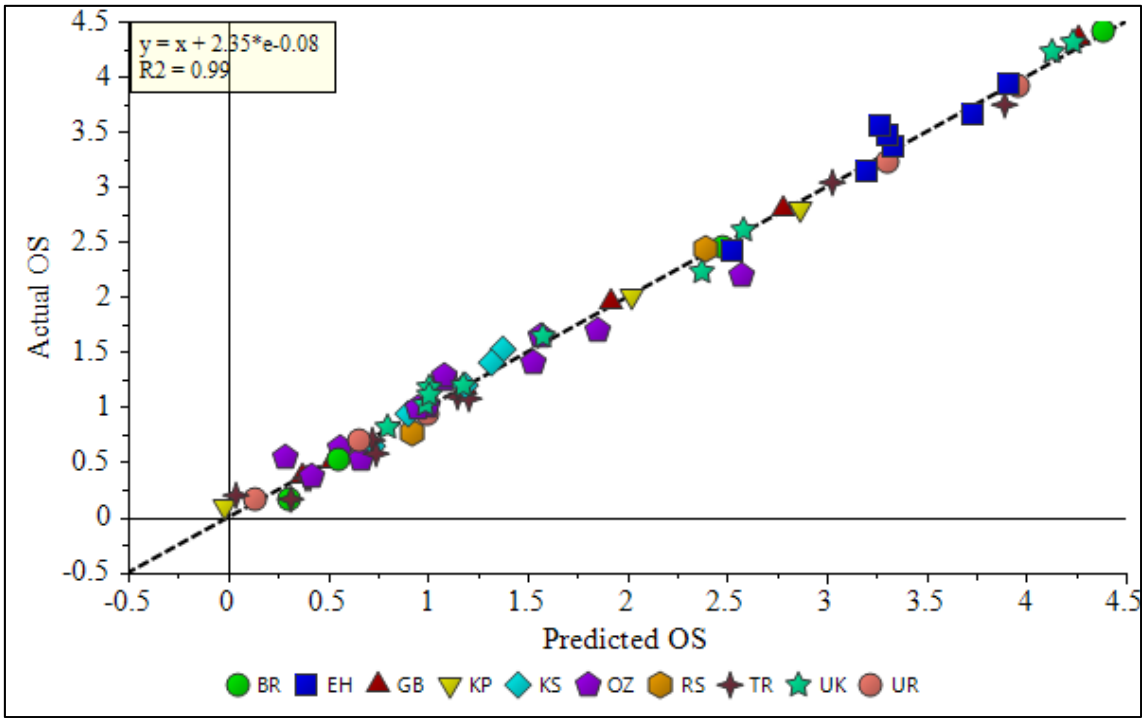


Figure 4.23. Plot of actual vs predicted OS (h^{-1}) obtained from PLS analysis using FTIR spectra

Table 4.15. Statistical results of PLS model for prediction of oxidative stability from FTIR data

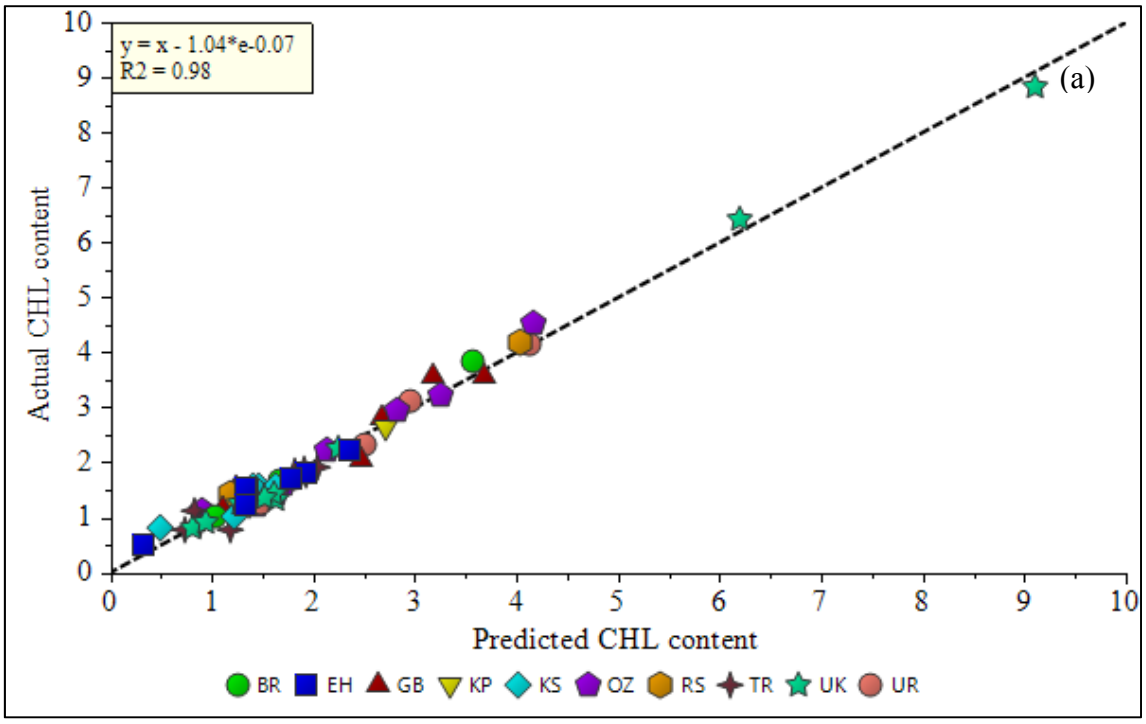
	R^2 (cal.)	R^2 (cv.)	RMSEC	RMSECV	Regression Equation
OS	0.99	0.81	0.11	0.68	y = x+2.35*e-0.08

Regression coefficient of calibration and cross-validation sets are determined as 0.99 and 0.81, respectively and these values indicate good prediction. RMSEC and RMSECV values are close to each other and also close to zero with the values of 0.11 and 0.86, orderly. Slope of the calibration curve is equal to 1 accounting for high reliability.

In the literature, FTIR has been used to evaluate the freshness of olive oil by Sinelli and coworkers (2007). Direct determination of peroxide value was studied in another study (Maggio et al., 2009). In the present study, quantitative determination of OS from IR spectra was tried and it was found out that prediction results were quite satisfactory. It is worth to emphasize that reference values of OS were obtained from Rancimat equipment and to best of our knowledge Rancimat originated OS data is predicted from IR spectroscopic measurement for the first time.

4.3.2. Prediction of Chlorophyll & Carotenoid Content From FTIR Profile

Chlorophyll and carotenoid values were predicted from FTIR profile and regression curves are given in Figure 4.24 (a) and (b) below.



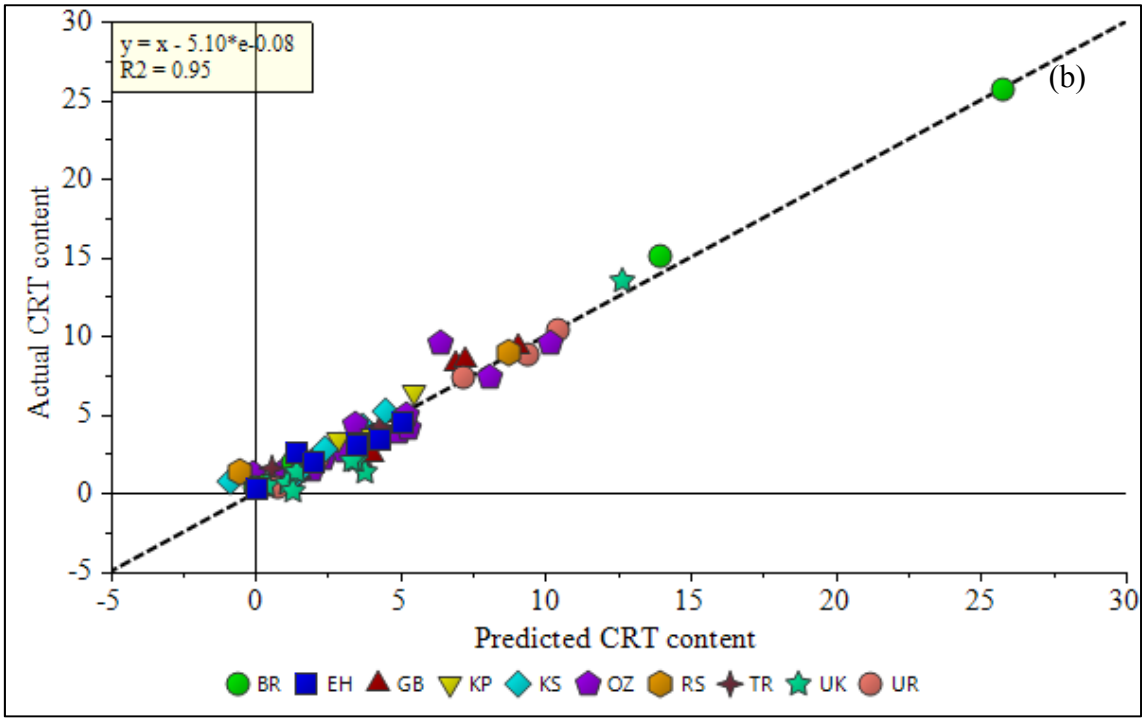


Figure 4.24. Plot of actual vs predicted (a) chlorophyll (mg/kg) (b) carotenoid content (mg/kg) obtained from PLS analysis using FTIR spectra

Statistical parameters of the chlorophyll and carotenoid PLS models are given in Table 4.16. According to this table chlorophyll calibration R^2 (0.98) is quite high while

cross-validation R^2 is in the range of approximate prediction limits (0.66-0.80). RMSEC (0.18) and RMSECV (0.95) values are lower than 1 but not very close to each other. Slope of calibration curve indicates quite reliable prediction. Carotenoid prediction parameters are not as good as chlorophyll due to the low value of regression coefficient of cross-validation (0.46) even though the value of calibration R^2 is high (0.95) meaning that reproducibility of the model is low. Other parameters like RMSEC and RMSECV are relatively high and distant to each other. It can be concluded that prediction of chlorophyll content form FTIR data is successful while prediction of carotenoid is not as good as chlorophyll. In the literature, chlorophyll and carotenoid contents were determined by different methods like chromatographic method (Gandul-Rojas et al., 2000) and UV spectrophotometric method (Mosquera et al, 1991); therefore, it is a new approach to predict chlorophyll and carotenoid contents from FTIR profile.

Table 4.16. Statistical results of PLS analysis for the prediction of chlorophyll and carotenoid content from FTIR data

	R^2 (cal.)	R^2 (cv.)	RMSEC	RMSECV	Regression Equation
Chl.	0.98	0.69	0.18	0.95	y = x-1.04*e-0.07
Crt.	0.95	0.46	0.93	3.01	y = x-5.10*e-0.08

4.3.3. Prediction of Fatty Acid Profile From FTIR Profile

The PLS regression analysis of FTIR data for the prediction of fatty acids resulted in a model with 4 PCs which explains 72.2 % of total variation with a predictive ability of 45.2% in overall model. To see the prediction power more clearly individual fatty acid components are analyzed. All the statistical parameters of calibration and cross validation for each model are given in Table 4.17.

Firstly, the most abundant fatty acid component existed in olive oil, oleic acid (C18:1n9c), is investigated. C18:1n9c was found in the range 68-70% in the present study. Predicted percentages plotted against the actual percentages of oleic acid for calibration model are given in Figure 4.25. According to this plot, R^2 value of oleic acid is found as 0.94 which indicates good prediction of calibration set but it is not enough for ultimate conclusion. Cross validation technique is used to see the predicted model validation and the result is quite successful with R^2 value of 0.81. RMSEC and

RMSECV values are also found as 0.44 and 0.97, respectively which are small and close to each other indicating there is no problem of over-fitting.

One of the important polyunsaturated fatty acids (PUFA), linoleic acid (C18:2n6c), is determined in the range of 7.87% to 15.13%. The observed vs predicted percentages of the calibration curve are shown in Figure 4.26. The statistical values of the model are satisfactory. Regression coefficients are determined as R^2 cal. = 0.97 and R^2 cv. = 0.91. RMSEC (0.36) and RMSECV (0.76) values show perfect fitting of the model. Slope of the calibration curve equals to 1 which is an indication of very reliable curve.

Table 4.17. Statistical results of the PLS regression model for the prediction of fatty acids from FTIR spectral data

	R^2 (cal.)	**R^2 (cv.)**	**RMSEC**	**RMSECV**	**Regression Equation**
C 16:0	0.87	0.70	0.35	0.55	y = x + 2.21*e-0.07
C 16:1	0.68	0.52	0.12	0.18	y = 0.97*x + 0.03
C 17:0	0.74	0.05	0.02	0.03	y = x + 3.83*e-0.09
C18:0	0.61	0.35	0.24	0.31	y = x - 1.33*e-0.07
C18:1n9c	0.94	0.81	0.44	0.97	y = x - 2.63*e-0.05
C18:2n6c	0.97	0.91	0.36	0.76	y = x -5.39*e-0.07
C20:0	0.65	0.19	0.03	0.05	y = x - 9.42*e-0.09
C20:1	0.39	0.23	0.11	0.12	y = x + 1.70*e-0.08
C18:3n3	0.09	0.00	0.08	0.08	y = x - 9.52*e-0.08
C22:0	0.61	-0.06	0.02	0.03	y = x - 7.07*e-0.09
SFA	0.91	0.79	0.35	0.61	y = x - 3.34*e-0.07
MUFA	0.94	0.82	0.45	0.93	y = x - 5.72*e-0.06
PUFA	0.97	0.91	0.36	0.77	y = x + 4.00*e-0.07

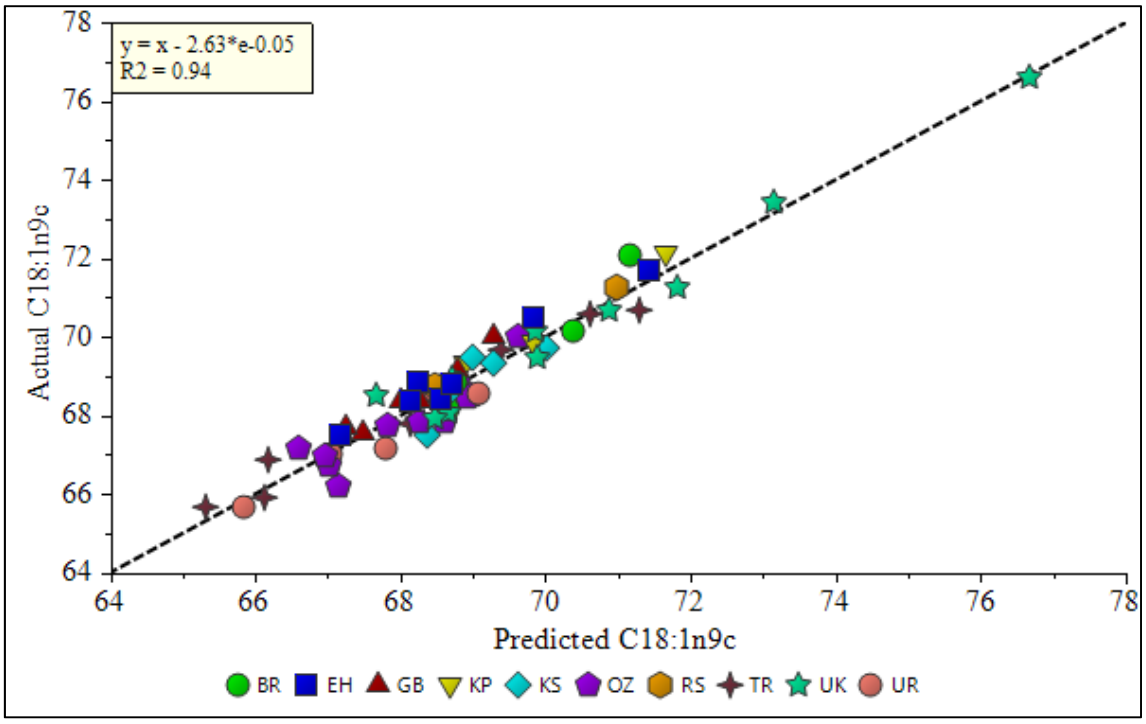


Figure 4.25. Plot of actual vs predicted oleic acid percentages obtained from PLS analysis using FTIR spectra

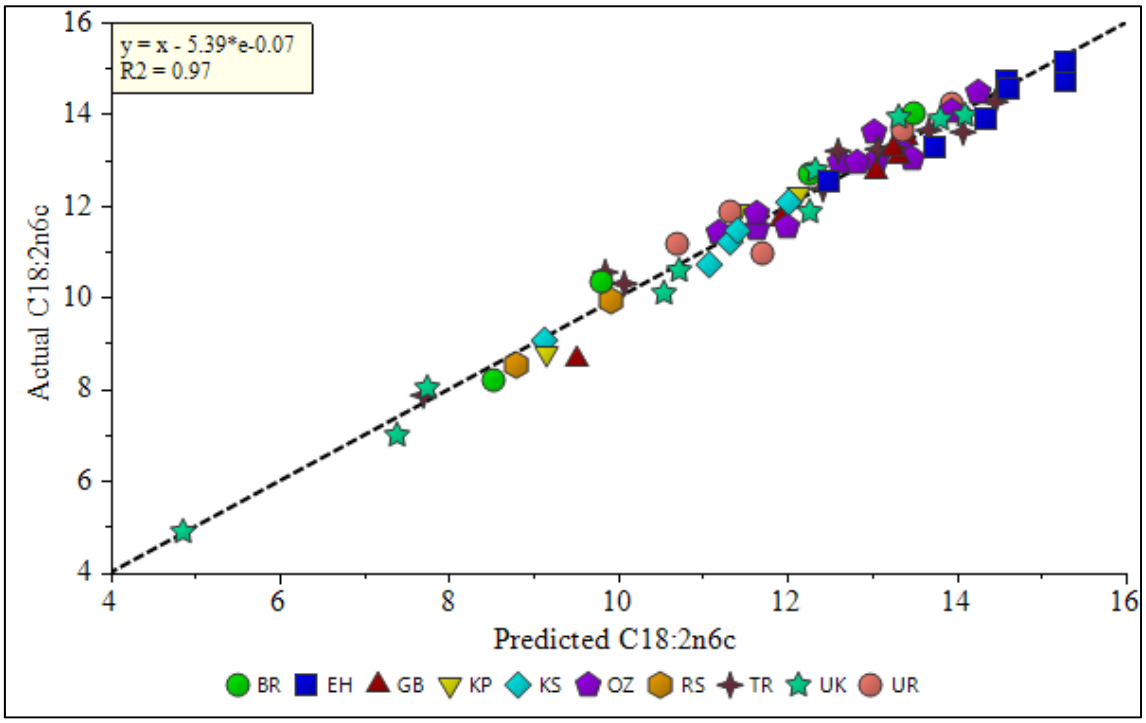


Figure 4.26. Plot of actual vs predicted linoleic acid percentages obtained from PLS analysis using FTIR spectra

Palmitic acid (C16:0) is the highest percentages of saturated fatty acids in the olive oil samples; therefore, it is important to predict palmitic acid content of olive oils accurately. The PLS regression model is given in Figure 4.27. PLS curve indicates that palmitic acid percentages could be detected with the regression coefficient value of 0.87 whereas cross-validation regression coefficient is 0.70 providing an approximate prediction on percentages of palmitic acid content. RMSEC and RMSECV values are really close to each other and small (0.35 and 0.55, orderly).

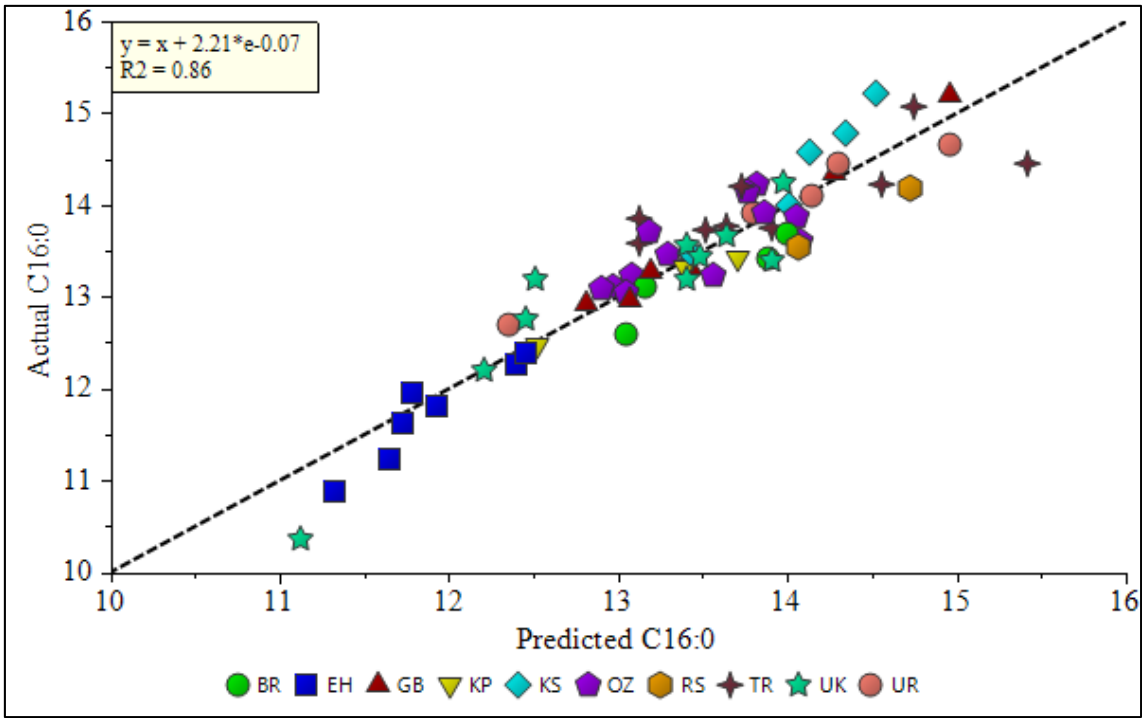


Figure 4.27. Plot of actual vs predicted palmitic acid percentages obtained from PLS analysis using FTIR spectra

MUFA, PUFA and SFA percentages are predicted from FTIR data with the perfect R^2 calibration value and the rest of the parameters are in the range of good prediction. In Figure 4.28, PLS regression plot for MUFA percentages are constructed. The rest of the fatty acids like C16:1 and C18:0 PLS results provide prediction to some extent whereas C20:0, C20:1, C18:3n3, C22:0 do not have good prediction models. In summary, higher amount of fatty acids have higher R^2 cal., and R^2 cv. while their RMSEC and RMSECV values are quite low. Oleic, linoleic and palmitic acids as individual fatty acids and MUFA, PUFA and SFA as combination of defined fatty acid groups could be predicted well from the FTIR data which is in accordance with the findings of Galtier and coworkers (2008). Mailer (2004) also found that fatty acids at

high concentrations had been predicted well on contrary to low concentration ones. These findings are also supported by the work of Gurdeniz et al. (2010) in which stearic, oleic and linoleic acids are predicted successfully as in the present case. It was also reported successful predictions of stearic, arachidic and linolenic acids in the same study.

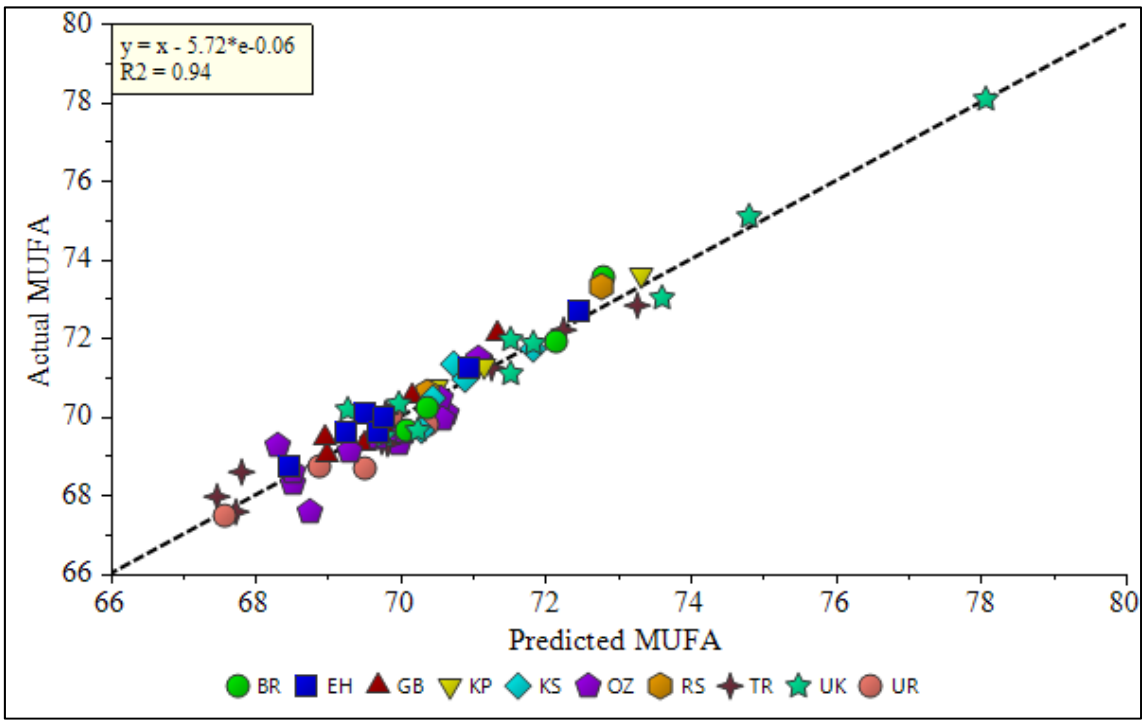


Figure 4.28. Plot of actual vs predicted MUFA content percentages obtained from PLS analysis using FTIR spectra

4.3.4. Prediction of Phenolic Compounds (TPC Included) from FTIR Profile

The PLS regression analysis using FTIR data for the prediction of phenolic compounds and TPC resulted in a model with 7 PCs which explains 64.7% of total variation with a low predictive ability of 6.14% in overall model. To see prediction power of FTIR spectra on each variable, individual phenolic compound PLS statistics are examined and they are provided in Table 4.18.

According to this table, the best prediction among the phenolic compounds is observed for p-coumaric acid with quite well R^2 calibration value 0.84 and R^2 cross-validation value of 0.28 which indicates poor validation of the model but it could be still

assumed that the model provides an accurate prediction to some extent. This is also supported by the values of small differences between RMSEC (0.51) and RMSECV (1.10). Plot of the regression model is given in Figure 4.29.

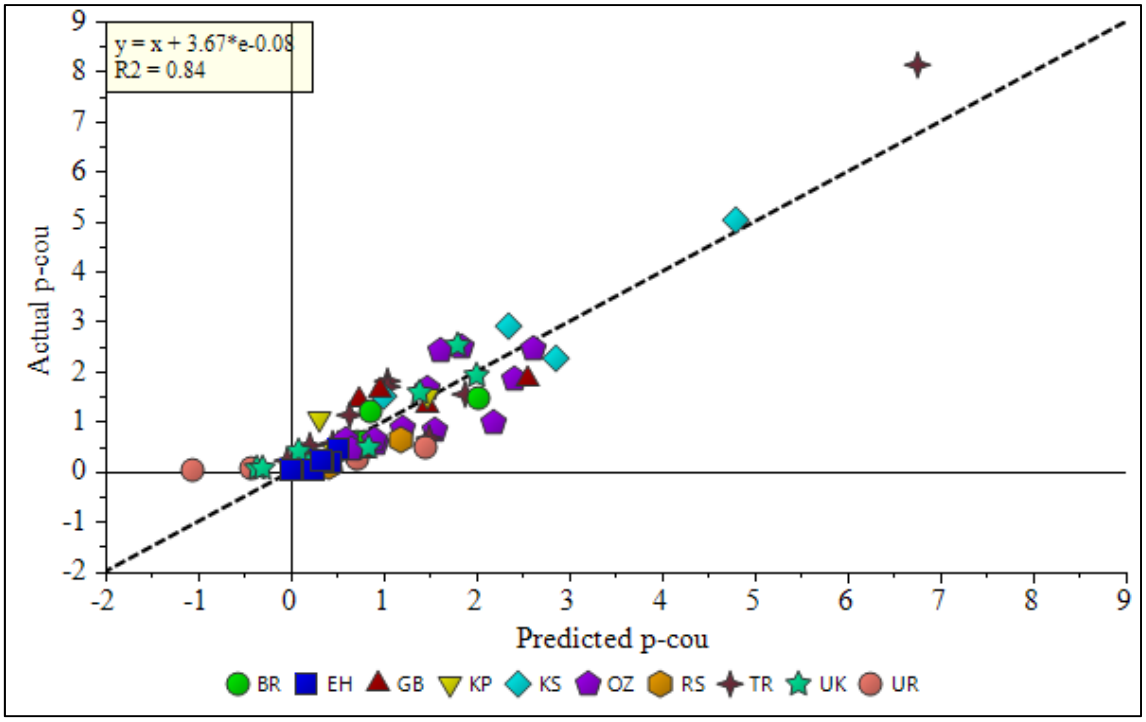


Figure 4.29. Plot of actual vs predicted p-coumaric acid content (mg/kg) obtained from PLS analysis using FTIR spectra

Table 4.18. Statistical results of the PLS regression model for the prediction of phenolic compounds and TPC from FTIR spectral data

	R^2 (cal.)	R^2 (cv.)	RMSEC	RMSECV	Regression Equation
TPC[1]	0.79	0.02	28.31	59.26	y = x + 2.25*e-0.06
Hxty[2]	0.83	0.22	2.72	5.87	y = x + 1.45*e-0.07
Tyrs[3]	0.74	0.17	5.15	9.14	y = x +1.86*e-0.07
4-Hypa[4]	0.46	-0.05	0.61	0.81	y = x - 1.54*e-0.08
3-Hypa[5]	0.65	-0.12	0.23	0.39	y = x - 2.72*e-0.08
Vna[6]	0.76	0.23	0.24	0.40	y = x + 2.06*e-0.08
Sya[7]	0.31	-0.16	0.05	0.06	y = x - 9.87*e-0.09
Cina[8]	0.74	0.11	0.04	0.07	y = x - 1.54*e-0.08
Cfa[9]	0.72	0.10	0.06	0.10	y = x - 4.74*e-0.09
Vnl[10]	0.46	-0.02	0.13	0.18	y = x - 8.39*e-0.09
P-cou[11]	0.84	0.28	0.51	1.10	y = x - 3.67*e-0.08
Apig[12]	0.42	0.07	0.87	1.06	y = x - 3.31*e-0.08
Lut[13]	0.69	0.06	0.31	0.53	y = x + 3.59*e-0.09

[1] total phenol content, [2] hydroxytyrosol, [3] tyrosol, [4] 4-hydroxybenzoic acid, [5] 3-hydroxybenzoic acid, [6] vanillic acid, [7]syringic acid, [8] cinnamic acid, [9] caffeic acid, [10] vanillin, [11] p-coumaric acid, [12] apigenin, [13] luteolin

It is worth to emphasize the good predictability of hydroxtyrosol content of olive oil is crucial due to its important contribution in olive oil oxidative stability and also its association with positive effect on health (Nan et al., 2014; Carrasco-Pancorbo et al., 2005b). Furthermore, the amount of hydroxtyrosol can be specifically mentioned on olive oil labels which can make product more remarkable. In the present study, statistical summary for the PLS model constructed for hydroxtyrosol have R^2 cal. (0.83), R^2 cv. (0.22) and RMSEC (2.72), RMSECV (5.87) revealing an average prediction ability on hydroxytyrosol. These values are not very good but still it can be useful to have an idea about hydroxtyrosol content of the samples in a shorter analysis time compared to chromatographic techniques. Prediction plot is given in Figure 4.30 below.

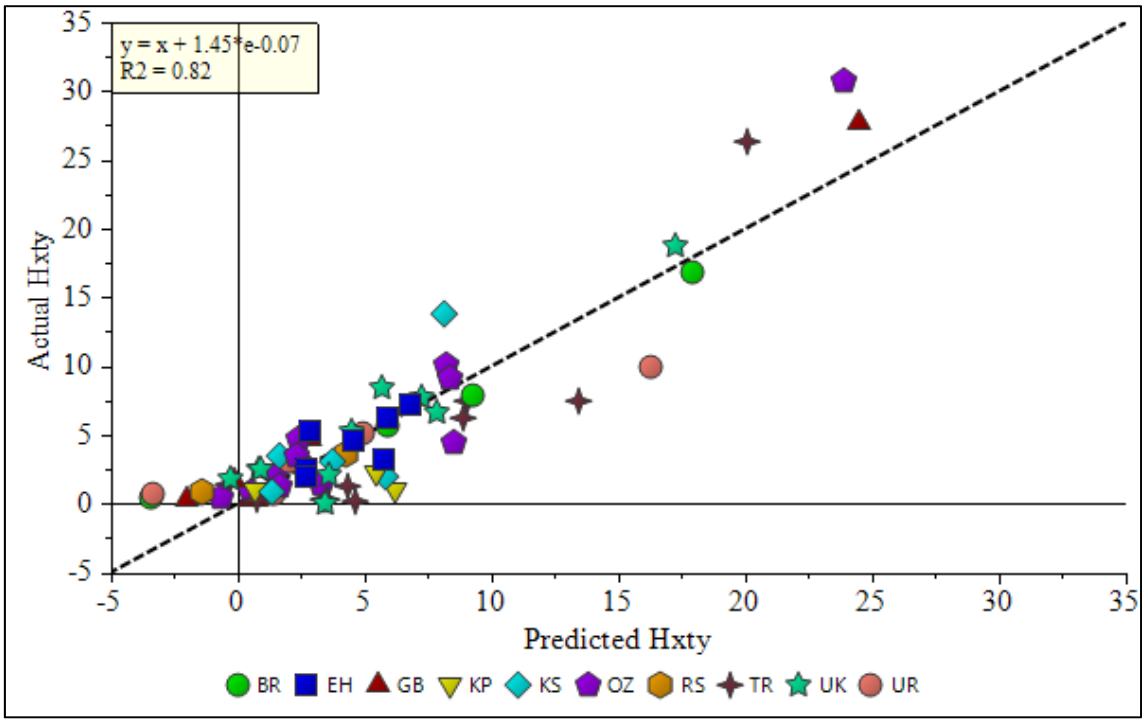


Figure 4.30. Plot of actual vs predicted hydroxytyrsol content (mg/kg) obtained from PLS analysis using FTIR spectra

Tyrosol, vanillic acid, cinnamic acid, and caffeic acid are not predicted as good as hydroxytyrosol and p-coumaric acid due to the low values of R^2 cal. 0.74, 0.76, 0.74 and 0.72, respectively. In addition, R^2 cv. values for these compounds are also low (0.17, 0.23, 0.11, and 0.10). For the rest of the phenolic compounds there is no good prediction observed at all. TPC value is also tried to be predicted from FTIR spectrum but the statistical values are not very promising while the regression coefficient for calibration is alright (0.79), but the cross-validation value is really low with the value of 0.02.

In the literature, IR spectrum was used to determine TPC and phenolic compounds in olive oil directly and indirectly. As an example of indirect detection Bellincontro and coworkers (2012) used near infrared (NIR) Acousto Optically Tunable Filter (AOTF) spectroscopy to determine TPC and some important phenolic compounds in olive fruit like oleuropein, verbascoside, and 3,4-DHPEA-EDA. As a result of mentioned study promising result were obtained. In another study, Yıldırım (2009) found that TPC value of olive oil could be predicted from FTIR successfully. To the best of our knowledge there is no study that determines phenolic compounds in olive oil directly. In the present study, it was aimed to find a correlation between IR spectrum and phenolic compounds, and average prediction was observed for p-coumaric acid and

hydroxtyrosol whereas tyrosol, vanillic acid, cinnamic acid and caffeic acid were not predicted as good as p-coumaric acids and hydroxytyrosol. Also, TPC was not determined as good as in Yıldırım study (2009). For the rest of phenolic compounds no good prediction was observed.

4.3.5. Prediction of Oxidative Stability from Various Chemical Parameters

In this part, the main aim is to observe the effect of individual components of fatty acids, phenolic substances (including TPC), chlorophyll and carotenoid contribution on oxidative stability using PLS regression and by monitoring the variable influence on the projection (VIP) values at the same time; therefore, finding out any possible relation between overall chemical parameters (fatty acid, phenolic compounds, TPC, chlorophyll and carotene) and oxidative stability.

PLS regression model is constructed in Figure 4.31 which explains 64% of the total variation with 13.7 % predictive ability. The statistical results are given in Table 4.19. In the light of this information, it was seen that R^2 cal. (0.64) and R^2 cv. (0.14) provide slight prediction from chemical data. Also, close root mean square error values of calibration (0.77) and cross validation (1.34) indicates that there is no over fitting. The reason of low prediction power could be one of the major oxidative stability contributor tocopherols especially α-tocopherol was not determined in the present study (Blekas et al., 1995).

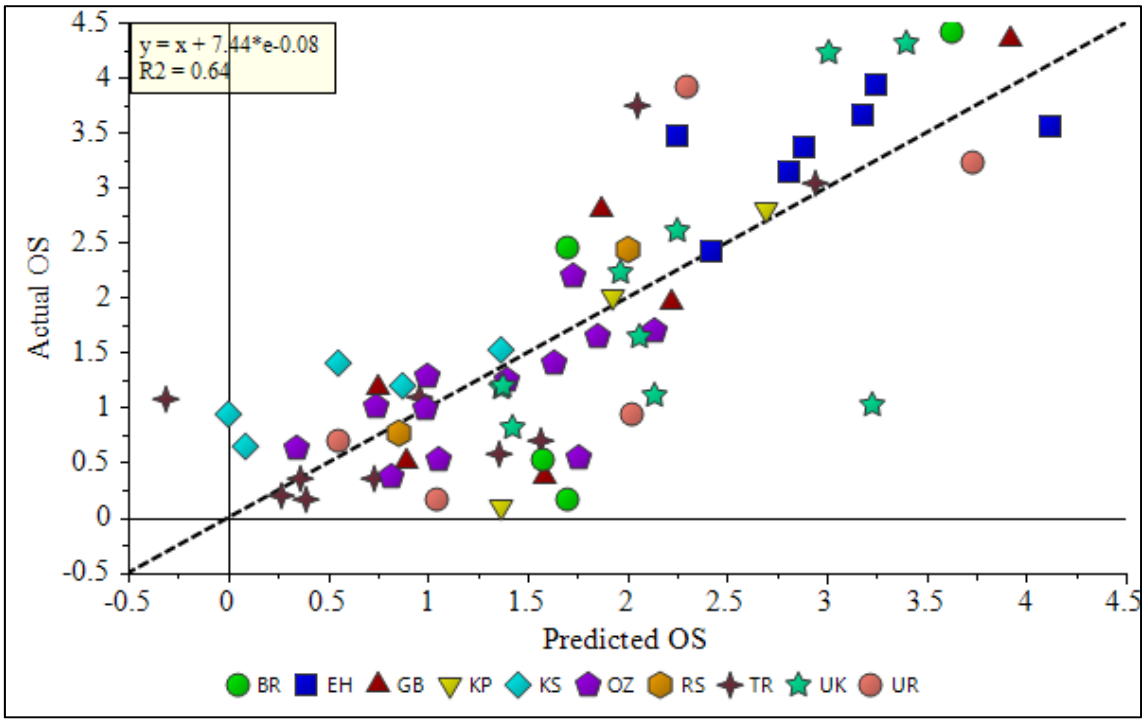


Figure 4.31. Plot of actual vs predicted OS (h^{-1}) obtained from PLS analysis using various chemical parameters

Table 4.19. Statistical results of the PLS regression model for the prediction of OS from various chemical parameters

	R^2 (cal.)	R^2 (cv.)	RMSEC	RMSECV	Regression Equation
OS	0.64	0.14	0.77	1.34	y = x + 7.44*e-0.08

Evaluation of VIP values gives an idea about the most important contributor to oxidative stress and it is accepted that for a variable to be effective on prediction, its VIP value must be higher or close to threshold value of 1. According to Figure 4.32 the most influential parameter is detected as palmitic acid, with the VIP value of 1.83. Palmitic acid is the saturated fatty acid with the highest percentages in olive oil and is known for its stability against oxidative stress. For vanillic acid, cinnamic acid and hydroxytyrosol similar VIP values are observed in descending order of 1.56, 1.51, 1.50, respectively. According to Carrasco-Pancorbo and coworkers (2005b) hydroxtyrosol has one of the highest anti-oxidant power (AOP) with other phenols like deacetoxy oleuropein aglycon and oleuropein aglycon. The present study also confirms the importance of hydroxtyrosol on oxidative stability with the VIP value of 1.50. VIP values of palmitoleic and p-coumaric acids are close to each other with values of 1.40

and 1.29, respectively. Caffeic acid, apigenin, tyrosol, eicosenoic acid, and total phenol content have VIP values in descending order of1.08, 1.03, 0.98, 0.96, and 0.92 and these values could be still considered as significant. ,. Rest of the parameters have lower VIP values and the variable effects become smaller and insgnificant.

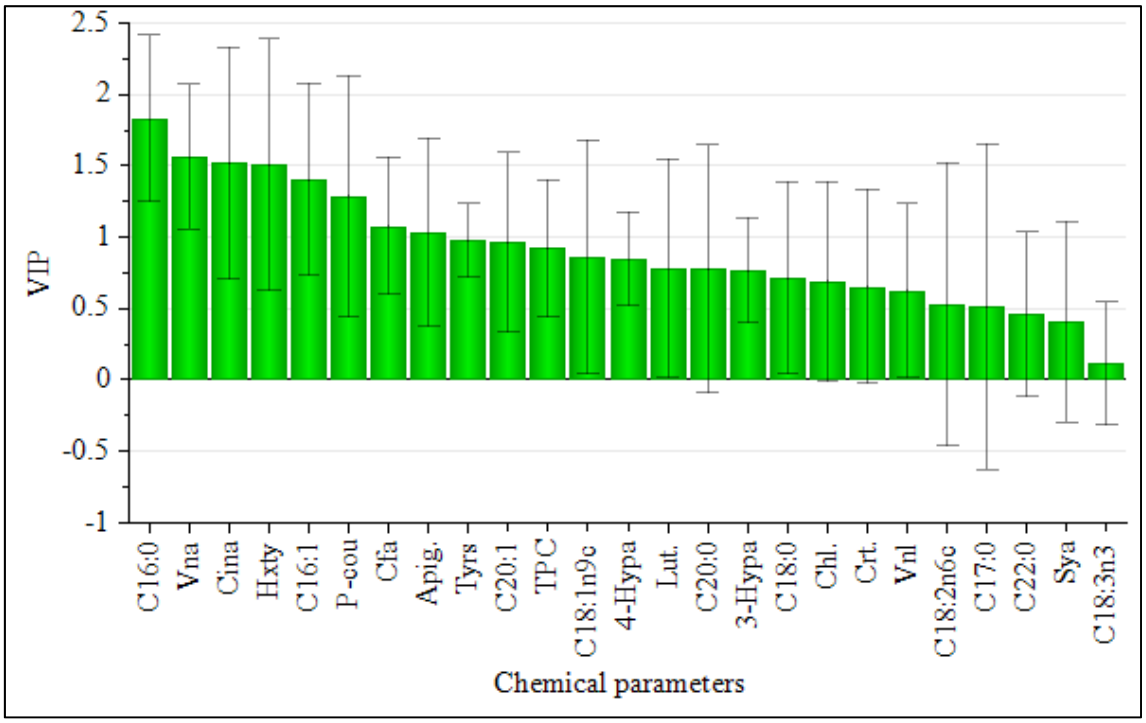


Figure 4.32. VIP values of PLS regression of OS from chemical parameters

CHAPTER 5

CONCLUSIONS

In the present study, geographical classification of olive oils from nine distinct locations of Karaburun Peninsula is investigated by using spectroscopic data and several chemical parameters in combination with chemometric techniques. Chemical parameters are carotene and chlorophyll content, oxidative stability, phenolic composition and fatty acid profile of olive oils. FTIR spectroscopy was also used to visualize mid-IR spectra of olive oil. Data was analyzed with multivariate statistical analysis techniques (PCA and PLS) to investigate the differentiation of olive oils with respect to geographical locations where olives come from and also to predict several chemical parameters of olive oils from FTIR spectroscopy.

PCA results were plotted to see the efficiency of each analysis on olive oil classification with respect to geographical location. Fatty acid profile did not provide much differentiation. The reason for this could be the use of only one variety, Erkence, in this study and the effect of cultivar is the most dominant factor in the olive oil classification with respect to fatty acid composition. However, still a minor effect of geographical location was observed even in a narrow study area (D'Imperio, et al. 2007). Differentiation with respect to phenolic content also explained small variation. As a rapid and reliable way of geographical classification FTIR methodology was also used to distinguish olive oils. Results were quite promising compared to other analyses. Moreover, second derivative of IR spectra improved classification. As a result, it can be concluded that the use of FTIR spectra was successful in classification purposes for even the same variety from close geographic area while classification power for fatty acid profile and phenolic compounds were not as not good as IR spectra.

PCA was also run using all chemical parameters (chlorophyll and carotenoid, OS, TPC, phenolic compounds and fatty acid profile) except FTIR data; however, differentiation was not better than the FTIR case.

Furthermore, correlations between FTIR profile and chemical parameters were set-up using PLS analysis. Prediction models for some fatty acids like oleic, linoleic, palmitic acids and MUFA, PUFA and SFA are robust with higher R^2 cal., R^2 cv., and

lower RMSEC, RMSECV. Oxidative stability and chlorophyll content are predicted perfectly while carotenoid content determination is not that reliable using FTIR spectroscopy. PLS models of some phenolic compounds and TPC from IR spectra are also examined and as a result p-coumaric acid and hydroxtyrsol are predicted to some extent while for the model for TPC is not very good. Apart from these, oxidative stability model developed from some chemical parameters (TPC, phenolic compounds, fatty acid content and chlorophyll and carotenoid) provided an approximate prediction with R^2 cal. value of 0.64. Moreover, VIP values reveal the most important contributor on oxidative stability of olive oil; therefore, palmitic, vanillic and cinnamic acid, hydroxytyrosol, palmitoleic and p-coumaric acids, caffeic acid, apigenin, tyrosol, eicosenoic acid, and total phenol content, are found as the most important contributors to oxidative stability in decrasing power in the studied case. To sum up, FTIR profile has high potential to predict the amount of some important chemical composition parameters in olive oil like major fatty acids, some phenolic compounds and some quality parameters like oxidative stability and chlorophyll.

The results of present work reveal the importance of infrared spectroscopic methods with numerous application areas like quantification of various chemical parameters, authenticity studies and geographical classification providing promising results. However, the high precision power of chromatographic methods should not be missed and these methods could still be used in compensation for conflicted results of spectroscopic methods. While promising, the reported results were the output of a single harvest year which can be improved by sampling over multiple years of harvest.

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APPENDIX A

EXPERIMENTAL DATA

Table A.1. Total phenol content, chlorophyll and caretonoids content, oxidative stablity of all regions

	TPC	CHL.	CRT.	OS
KP1	295.35	1.00	6.43	0.10
OZ2	334.13	1.04	3.88	0.54
UR3	252.15	4.16	10.38	0.93
OZ4	251.31	1.44	1.47	1.25
KS5	284.81	0.83	0.73	1.52
UR7	278.44	2.31	8.86	0.16
GB8	323.41	2.80	8.21	0.37
BR9	323.14	3.86	15.12	0.16
OZ10	285.24	1.31	1.47	1.01
GB11	238.64	1.53	3.52	0.51
KS12	295.11	1.56	4.19	0.65
TR13	362.34	0.76	1.84	0.35
OZ14	474.74	1.11	1.16	1.70
OZ16	289.21	1.22	2.17	0.63
TR17	288.57	1.90	4.21	0.17
TR18	236.36	1.12	1.78	0.35
OZ19	305.55	1.62	4.34	0.53
TR20	290.94	0.78	3.61	0.19
TR21	346.90	0.88	1.00	0.70
RS22	281.34	1.45	1.31	0.77
TR23	342.27	1.79	1.79	3.75
GB24	234.66	3.57	8.45	1.96
UR25	254.86	1.44	2.12	3.92
OZ26	235.26	3.22	9.53	2.19
TR27	397.07	1.88	2.99	3.04
BR28	291.17	1.72	0.54	2.45
TR29	239.23	1.84	1.57	1.09

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Table A.1. (cont.) Total phenol content, chlorophyll and caretonoids content, oxidative stablity of all regions

	TPC	CHL.	CRT.	OS
GB30	209.90	1.15	3.31	1.18
OZ31	235.42	1.25	4.13	0.37
BR32	285.39	1.02	1.97	0.52
OZ33	225.98	1.44	2.69	1.29
TR34	314.99	1.30	1.29	1.07
KS35	214.28	1.57	5.19	1.19
UR36	392.28	1.25	0.31	3.23
TR37	192.31	1.24	1.14	0.57
UR38	237.83	3.11	7.37	0.70
KP40	226.46	1.65	3.64	2.01
KS41	254.17	1.01	1.17	0.94
BR42	409.98	1.69	0.38	4.41
KP43	277.29	2.64	3.36	2.80
OZ44	245.85	2.21	4.96	1.40
GB45	242.16	3.58	9.27	2.80
OZ46	293.50	4.53	9.58	1.65
UK47	259.45	1.34	1.29	4.23
KS48	254.61	1.56	2.74	1.40
OZ49	280.37	2.95	7.32	0.99
UK50	217.12	1.44	2.95	0.82
GB51	491.95	2.05	2.41	4.35
UK52	263.99	6.44	13.52	2.23
UK53	268.40	8.84	25.63	1.64
UK54	349.59	1.26	0.52	4.31
RS55	195.77	4.19	8.96	2.43
UK56	260.96	0.81	0.59	1.18
UK57	293.34	1.37	2.02	2.61
UK58	188.46	2.26	1.39	1.02
UK60	241.31	1.36	2.04	1.19
UK61	315.30	0.90	0.11	1.11
EH62	261.30	1.49	3.15	2.41
EH63	256.79	1.80	3.43	3.36
EH64	211.09	2.23	4.49	3.65
EH65	216.56	1.55	2.58	3.46
EH66	254.30	1.70	3.00	3.56
EH67	257.97	1.22	1.89	3.14
EH68	247.77	0.51	0.26	3.94

Table A.2. Individual phenolic compounds of all region at 280 nm.

	Hxty	Tyrs	4-Hypa	3-Hypa	Vna	Sya	Cina	O-Cou
KP1	0.98	11.50	0.23	0.40	0.71	nd	nd	nd
OZ2	10.02	17.58	0.30	0.67	1.07	0.10	0.13	nd
UR3	5.06	5.22	0.49	0.65	0.82	0.14	nd	nd
OZ4	2.07	3.58	0.18	0.29	0.52	nd	0.02	nd
KS5	13.77	23.68	0.65	0.50	0.94	0.10	nd	0.04
UR7	0.66	1.67	0.17	0.22	0.39	0.02	nd	nd
GB8	0.37	5.52	0.42	0.40	0.49	0.06	0.06	nd
BR9	0.38	10.83	1.37	0.88	1.13	0.15	0.04	nd
OZ10	0.82	4.75	0.94	0.97	1.62	0.10	0.23	nd
GB11	0.29	5.97	0.75	0.81	0.93	0.13	0.07	nd
KS12	1.94	10.12	0.80	0.73	1.61	0.07	0.16	nd
TR13	0.11	5.51	0.97	0.27	1.71	0.10	0.23	nd
OZ14	30.72	31.18	2.14	0.94	1.65	0.16	0.03	nd
OZ16	3.03	11.68	1.87	0.80	1.26	0.09	0.10	nd
TR17	0.33	5.76	1.04	0.76	0.96	0.09	0.09	0.16
TR18	1.26	14.72	0.76	0.69	0.63	0.07	0.03	0.03
OZ19	9.07	14.29	0.58	0.91	1.60	0.07	0.02	nd
TR20	0.29	8.01	1.79	2.27	2.87	0.14	0.41	0.30
TR21	7.41	20.34	0.74	0.54	0.80	0.10	0.03	nd
RS22	3.63	4.44	0.84	0.33	0.88	0.38	0.01	0.01
TR23	6.26	5.84	0.82	0.55	0.49	0.13	nd	nd
GB24	4.78	7.58	0.42	0.31	0.42	0.03	0.02	nd

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Table A.2.(cont.) Individual phenolic compounds of all region at 280 nm.

	Hxty	Tyrs	4-Hypa	3-Hypa	Vna	Sya	Cina	O-Cou
UR25	3.11	1.22	0.14	0.08	0.19	0.05	nd	nd
OZ26	1.30	7.26	0.73	0.53	0.68	0.06	0.02	nd
TR27	26.31	40.83	0.80	1.17	0.90	0.11	0.05	nd
BR28	5.63	5.70	0.20	0.63	1.81	0.10	0.16	nd
TR29	1.42	4.03	0.26	0.32	0.50	0.04	0.06	nd
GB30	1.75	3.47	0.31	0.12	0.57	0.04	0.05	nd
OZ31	1.38	5.35	0.55	0.28	0.92	0.06	0.08	nd
BR32	7.95	18.52	1.30	0.68	0.98	0.08	0.05	nd
OZ33	4.67	10.64	0.35	0.40	0.71	0.05	nd	nd
TR34	7.45	44.19	5.99	0.98	1.11	0.08	0.03	nd
KS35	3.10	7.37	1.35	0.65	1.19	0.11	0.29	nd
UR36	9.95	31.74	nd	0.45	0.24	0.07	0.08	nd
TR37	0.32	2.85	0.19	0.21	0.23	0.03	0.02	nd
UR38	0.75	3.34	0.64	0.50	0.62	0.08	0.06	nd
KP40	1.00	5.80	0.89	0.59	0.75	0.05	0.07	nd
KS41	3.50	7.59	0.79	0.34	1.16	0.05	0.15	nd
BR42	16.88	30.49	0.55	0.67	0.76	0.07	0.04	nd
KP43	2.30	10.79	0.47	0.34	0.25	0.09	0.01	nd
OZ44	0.48	15.25	0.74	0.73	0.41	0.04	0.02	nd
GB45	0.27	3.89	0.75	0.69	0.95	0.18	0.17	nd
OZ46	4.45	5.35	0.18	0.19	0.52	0.05	nd	nd

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Table A.2.(cont.) Individual phenolic compounds of all region at 280 nm.

	Hxty	Tyrs	4-Hypa	3-Hypa	Vna	Sya	Cina	O-Cou
UK47	5.31	11.63	0.52	0.23	0.34	0.05	nd	nd
KS48	0.91	4.52	1.02	0.59	1.08	0.07	0.01	nd
OZ49	3.48	12.33	1.43	0.73	0.93	0.07	0.10	nd
UK50	7.68	8.05	0.20	0.27	0.57	0.07	nd	nd
GB51	27.66	24.45	0.24	0.51	0.14	0.03	0.02	nd
UK52	2.52	5.29	0.29	0.63	0.65	0.08	0.13	0.30
UK53	1.79	4.62	0.32	0.47	1.29	0.04	0.08	0.09
UK54	18.74	38.65	1.19	1.37	0.47	0.04	0.02	nd
RS55	0.85	3.79	0.44	0.37	0.69	0.04	0.13	0.15
UK56	2.08	3.74	0.31	0.15	0.37	0.05	nd	nd
UK57	6.63	24.81	1.56	1.88	1.31	0.09	0.11	nd
UK58	0.09	0.73	0.18	0.19	0.22	0.01	nd	nd
UK60	2.51	5.91	0.55	0.63	0.83	0.05	0.04	0.05
UK61	8.38	16.14	1.42	1.15	0.84	0.16	0.02	0.04
EH62	4.57	5.85	0.37	0.45	0.58	0.13	0.03	nd
EH63	7.25	10.03	0.27	0.71	0.51	0.16	0.03	nd
EH64	2.56	4.72	0.26	0.54	0.34	0.05	0.05	0.01
EH65	1.98	3.83	0.20	0.43	0.32	0.05	0.04	nd
EH66	6.16	11.33	0.41	0.70	0.47	0.08	0.03	nd
EH67	5.33	8.29	0.27	0.75	0.49	0.07	0.06	nd
EH68	3.25	4.45	0.27	0.44	0.31	0.06	0.02	nd

Table A.3. Individual phenolic compounds of all region at 320 nm.

	2-3 Dhxyb	Chla	Cfa	Vnl	P-Cou	Apig	Lut
KP1	nd	nd	nd	0.13	1.04	2.85	nd
OZ2	nd	nd	0.08	1.14	1.64	0.60	0.18
UR3	nd	nd	0.05	0.27	0.51	0.58	nd
OZ4	nd	nd	0.16	0.60	0.88	0.49	0.69
KS5	nd	nd	0.15	0.06	0.28	0.21	0.07
UR7	nd	nd	0.01	0.33	0.24	0.83	nd
GB8	nd	nd	0.07	0.05	1.42	2.79	0.17
BR9	0.10	nd	0.06	0.09	0.60	1.80	0.09
OZ10	nd	nd	0.23	0.32	2.43	2.58	0.16
GB11	nd	nd	0.02	0.10	0.39	0.65	nd
KS12	nd	nd	0.19	0.24	2.25	1.97	1.59
TR13	nd	nd	0.09	0.04	1.56	0.74	0.38
OZ14	nd	0.05	0.35	0.26	0.96	0.76	nd
OZ16	nd	nd	0.27	0.01	2.48	nd	0.06
TR17	nd	nd	0.04	0.04	1.13	1.13	0.07
TR18	nd	nd	0.06	nd	0.57	1.59	nd
OZ19	nd	0.03	0.29	0.11	0.84	1.92	nd
TR20	nd	nd	0.45	0.12	8.13	5.29	nd
TR21	nd	nd	0.09	0.07	0.55	0.04	0.04
RS22	nd	nd	0.08	0.14	0.11	0.60	0.05
TR23	nd	nd	0.03	0.10	0.20	0.15	0.05
GB24	nd	nd	0.07	0.10	0.36	2.24	nd

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Table A.3.(cont.) Individual phenolic compounds of all region at 320 nm.

	2-3 Dhxyb	Chla	Cfa	Vnl	P-Cou	Apig	Lut
UR25	nd	nd	0.01	0.07	0.03	0.06	nd
OZ26	nd	0.02	0.10	0.06	0.57	1.61	nd
TR27	nd	nd	0.11	0.06	1.81	0.06	0.02
BR28	nd	nd	0.09	0.26	1.46	1.65	nd
TR29	nd	nd	0.09	0.10	1.71	3.27	0.17
GB30	nd	nd	0.05	0.05	1.31	1.04	0.04
OZ31	nd	nd	0.24	0.31	1.84	2.22	0.88
BR32	0.04	0.01	0.09	0.06	0.59	0.62	0.03
OZ33	nd	nd	0.09	0.38	0.63	1.56	nd
TR34	0.05	0.05	0.18	nd	0.68	0.91	0.02
KS35	nd	nd	0.60	0.42	5.01	0.05	0.02
UR36	nd	nd	0.05	nd	0.06	0.07	nd
TR37	nd	nd	0.02	0.06	0.54	1.19	0.05
UR38	nd	nd	0.04	0.14	0.48	1.12	0.06
KP40	nd	nd	0.11	0.10	1.49	2.29	0.17
KS41	nd	nd	0.30	0.11	2.89	0.78	0.19
BR42	nd	0.06	0.10	0.12	1.19	1.03	nd
KP43	nd	nd	0.01	0.07	0.40	1.39	0.38
OZ44	nd	nd	0.04	0.30	0.45	0.87	nd
GB45	nd	0.02	0.11	0.26	1.84	3.68	0.47
OZ46	nd	nd	0.11	0.07	0.64	3.35	nd

(cont. on next page)

Table A.3.(cont.) Individual phenolic compounds of all region at 320 nm.

	2-3 Dhxyb	Chla	Cfa	Vnl	P-Cou	Apig	Lut
UK47	nd	nd	0.05	0.05	0.15	0.20	nd
KS48	nd	0.04	0.03	0.07	1.52	0.67	1.07
OZ49	nd	nd	0.10	0.14	2.40	3.23	nd
UK50	nd	nd	0.08	nd	0.19	1.14	0.84
GB51	nd	nd	0.08	0.04	1.63	2.17	0.23
UK52	nd	nd	0.12	0.52	1.59	2.29	nd
UK53	nd	nd	0.09	0.17	0.47	0.52	nd
UK54	nd	nd	0.05	0.02	0.20	0.08	nd
RS55	nd	nd	0.04	0.08	0.62	1.95	0.03
UK56	nd	nd	0.02	0.10	0.39	0.40	1.38
UK57	nd	nd	0.08	0.11	1.91	0.90	0.13
UK58	nd	nd	0.02	0.01	0.07	0.15	1.56
UK60	nd	nd	0.13	0.37	2.53	nd	0.42
UK61	nd	nd	0.04	nd	0.07	nd	2.55
EH62	nd	nd	0.10	0.16	0.44	nd	0.73
EH63	nd	nd	0.05	0.16	0.18	nd	0.81
EH64	nd	nd	0.01	0.06	0.18	nd	0.59
EH65	nd	nd	0.02	0.15	0.17	nd	1.63
EH66	nd	nd	0.03	0.11	0.04	nd	0.12
EH67	nd	nd	0.03	0.15	0.02	nd	0.16
EH68	nd	nd	0.01	0.08	0.23	0.66	1.80

Table A.4. Individual fatty acid compenents of all regions

	C8:0	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1n9c	C18:2n6c
KP1	0.03	nd	nd	13.42	0.75	0.13	2.80	69.33	11.87
OZ2	0.01	0.03	nd	13.24	0.67	0.16	3.23	67.85	12.95
UR3	nd	nd	nd	13.92	0.73	0.17	3.78	68.54	10.96
OZ4	nd	0.02	0.01	14.23	0.76	0.16	3.70	66.19	12.98
KS5	nd	nd	nd	14.79	1.41	0.13	3.42	69.70	9.06
UR7	0.05	nd	nd	14.66	0.77	0.24	3.79	67.03	11.16
GB8	0.02	0.02	0.03	13.31	0.73	0.15	2.90	67.52	13.46
BR9	0.02	nd	nd	13.43	0.89	0.16	3.57	72.07	8.21
OZ10	nd	0.02	0.01	14.14	0.90	0.14	2.73	68.51	11.52
GB11	nd	nd	nd	14.36	0.92	0.19	3.30	67.70	11.69
KS12	nd	nd	nd	13.99	1.10	0.13	2.69	69.47	10.70
TR13	0.02	nd	nd	15.07	1.42	0.15	3.09	70.70	7.87
OZ14	nd	nd	nd	13.12	0.64	0.14	2.62	68.31	13.58
OZ16	nd	nd	nd	13.60	0.66	0.15	2.78	67.17	13.25
TR17	nd	nd	nd	14.46	0.97	nd	2.82	65.69	13.63
TR18	0.03	nd	nd	14.20	0.83	0.15	2.77	65.90	14.26
OZ19	0.03	0.02	nd	13.87	0.93	0.14	3.04	68.84	11.40
TR20	0.03	nd	nd	14.23	0.88	0.15	2.75	69.66	10.55
TR21	0.04	nd	nd	13.72	0.80	0.14	2.60	67.79	13.21
RS22	0.02	nd	nd	14.18	1.00	0.20	3.94	68.74	9.94
TR23	nd	nd	nd	13.41	0.91	0.15	2.91	70.57	10.28
GB24	nd	nd	nd	12.92	0.65	0.15	2.73	69.14	12.72

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Table A.4.(cont.) Individual fatty acid compenents of all regions

	C8:0	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1n9c	C18:2n6c
UR25	nd	nd	nd	14.09	0.73	0.20	3.94	67.15	11.88
OZ26	nd	nd	nd	13.09	0.68	0.14	2.72	7nd	11.55
TR27	nd	nd	nd	13.86	0.76	0.13	2.49	67.84	13.24
BR28	nd	nd	nd	13.12	0.73	0.16	3.17	68.16	12.69
TR29	nd	nd	nd	13.74	0.86	0.16	2.82	66.88	13.61
GB30	nd	nd	nd	15.20	1.35	0.12	3.03	70.02	8.67
OZ31	0.01	nd	nd	13.92	0.79	0.14	3.16	68.46	11.82
BR32	0.02	nd	nd	13.70	1.01	0.11	2.93	70.16	10.34
OZ33	nd	nd	nd	13.46	0.71	0.14	2.69	66.74	14.49
TR34	nd	nd	nd	13.59	0.76	0.12	2.53	67.93	13.26
KS35	nd	nd	nd	14.58	1.13	0.13	2.68	68.56	11.19
UR36	nd	nd	nd	12.70	0.58	0.14	2.79	68.56	13.63
TR37	nd	nd	nd	13.77	0.94	0.15	2.63	68.39	12.35
UR38	nd	nd	nd	14.45	0.98	0.14	2.70	65.66	14.24
KP40	nd	nd	nd	12.46	0.68	0.14	2.83	69.87	12.23
KS41	nd	0.01	nd	15.22	1.33	0.14	2.60	67.50	11.45
BR42	nd	nd	nd	12.60	0.64	nd	2.62	68.85	14.01
KP43	nd	0.02	nd	13.31	0.81	0.09	3.24	72.14	8.77
OZ44	nd	0.02	nd	13.24	0.83	0.17	3.41	66.96	13.05
GB45	nd	nd	nd	13.27	nd	0.13	3.00	68.35	13.08
OZ46	0.01	0.01	nd	13.04	0.65	0.15	2.66	67.74	14.07

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Table A.4.(cont.) Individual fatty acid compenents of all regions

	C8:0	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1n9c	C18:2n6c
UK47	nd	nd	nd	13.20	1.05	0.15	3.70	73.42	6.99
KS48	nd	0.01	nd	13.49	0.82	0.12	2.42	69.33	12.06
OZ49	0.01	0.02	nd	13.71	0.87	0.14	2.75	67.85	12.95
UK50	0.01	0.01	nd	13.57	0.83	0.14	2.83	68.05	12.80
GB51	0.01	0.01	nd	12.97	0.64	0.13	2.85	68.37	13.22
UK52	0.01	0.01	nd	13.66	1.05	0.16	3.09	70.13	10.10
UK53	0.01	nd	nd	13.39	0.82	0.17	3.23	68.51	11.89
UK54	nd	nd	nd	13.44	0.89	0.17	3.68	69.46	10.57
RS55	nd	nd	nd	13.54	1.27	0.15	3.48	71.27	8.51
UK56	0.01	0.01	nd	14.25	1.14	0.16	3.54	71.25	8.01
UK57	nd	nd	nd	12.20	0.61	0.13	2.50	68.94	13.99
UK58	0.06	nd	nd	10.35	0.42	0.16	2.76	70.69	13.94
UK60	0.01	0.02	nd	13.19	0.71	0.14	2.66	67.91	13.91
UK61	0.01	nd	nd	12.76	0.92	0.17	3.21	76.59	4.90
EH62	nd	0.01	nd	12.26	0.51	0.14	2.79	68.84	13.88
EH63	nd	nd	nd	11.80	0.49	0.14	2.88	68.39	14.72
EH64	nd	0.02	nd	11.62	0.48	0.14	2.81	68.79	14.54
EH65	nd	nd	nd	11.96	0.52	0.15	2.70	68.36	14.74
EH66	nd	0.02	nd	10.88	0.42	0.14	2.88	71.69	12.51
EH67	nd	0.02	nd	12.38	0.52	0.13	2.72	67.51	15.13
EH68	nd	0.02	nd	11.23	0.13	0.13	2.89	70.48	13.26

Table A.5. Individual fatty acid compenents of all regions

	C20:0	C20:1	C18:3n3	C21:0	C20:2	C22:0	C20:3n6	C20:4n6	C24:0
KP1	0.44	0.69	0.31	nd	nd	0.13	nd	0.03	0.08
OZ2	0.47	0.79	0.32	0.02	nd	0.13	nd	0.03	0.10
UR3	0.54	0.77	0.29	0.02	0.04	0.15	nd	0.03	0.08
OZ4	0.48	0.64	0.29	0.02	nd	0.13	nd	0.04	0.12
KS5	0.40	0.62	0.25	nd	nd	0.12	nd	nd	0.10
UR7	0.63	0.95	0.32	nd	nd	0.18	nd	0.03	0.20
GB8	0.47	0.78	0.32	0.02	nd	0.14	nd	0.03	0.10
BR9	0.51	0.60	0.28	nd	nd	0.14	nd	0.04	0.09
OZ10	0.44	0.65	0.30	0.02	nd	0.12	nd	0.43	0.07
GB11	0.53	0.83	0.30	nd	nd	0.15	nd	0.04	nd
KS12	0.58	0.75	0.30	nd	nd	0.13	nd	0.03	0.15
TR13	0.49	0.69	0.28	nd	nd	0.13	nd	0.03	0.08
OZ14	0.42	0.66	0.31	nd	nd	0.12	nd	0.03	0.07
OZ16	0.46	1.44	0.33	nd	nd	0.15	nd	nd	nd
TR17	0.44	1.32	0.38	nd	nd	nd	nd	nd	0.30
TR18	0.44	0.82	0.32	nd	nd	0.12	0.04	0.04	0.08
OZ19	0.45	0.70	0.30	nd	nd	0.12	0.05	0.03	0.07
TR20	0.48	0.70	0.33	nd	nd	0.14	nd	0.04	0.08
TR21	0.41	0.76	0.31	nd	nd	0.12	nd	0.04	0.09
RS22	0.53	0.85	0.33	nd	nd	0.14	nd	0.05	0.08
TR23	0.46	0.73	0.34	nd	nd	0.13	nd	0.04	0.08
GB24	0.42	0.71	0.32	0.01	nd	0.13	nd	0.03	0.07

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Table A.5.(cont.) Individual fatty acid compenents of all regions

	C20:0	C20:1	C18:3n3	C21:0	C20:2	C22:0	C20:3n6	C20:4n6	C24:0
UR25	0.61	0.80	0.31	0.02	nd	0.15	nd	0.03	0.07
OZ26	0.43	0.79	0.30	0.04	nd	0.13	0.03	0.04	0.08
TR27	0.40	0.71	0.30	0.03	nd	0.11	nd	0.02	0.11
BR28	0.50	0.75	0.30	0.07	0.05	0.23	nd	0.02	0.06
TR29	0.46	0.86	0.34	nd	nd	0.14	nd	0.03	0.09
GB30	0.44	0.72	0.25	nd	nd	0.12	nd	0.03	0.06
OZ31	0.47	0.69	0.33	nd	nd	0.12	nd	0.03	0.08
BR32	0.45	0.74	0.29	nd	nd	0.12	nd	0.03	0.11
OZ33	0.42	0.84	0.29	nd	nd	0.13	nd	nd	0.07
TR34	0.38	0.85	0.48	nd	nd	0.10	nd	nd	nd
KS35	0.43	0.76	0.31	nd	nd	0.11	nd	0.02	0.08
UR36	0.45	0.67	0.38	nd	nd	0.11	nd	nd	nd
TR37	0.45	0.76	0.33	nd	nd	0.13	nd	0.03	0.08
UR38	0.46	0.82	0.29	nd	nd	0.13	0.03	0.03	0.08
KP40	0.47	0.72	0.35	nd	nd	0.14	nd	0.04	0.08
KS41	0.42	0.82	0.27	0.02	nd	0.10	0.01	0.03	0.09
BR42	0.34	0.70	0.24	nd	nd	nd	nd	nd	nd
KP43	0.45	0.65	0.29	0.02	nd	0.13	nd	0.02	0.07
OZ44	0.49	0.80	0.83	0.01	nd	0.12	nd	0.03	0.06
GB45	0.39	0.97	0.43	nd	0.09	0.09	nd	nd	0.19
OZ46	0.40	0.74	0.31	0.02	nd	0.11	nd	0.02	0.08

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Table A.5.(cont.) Individual fatty acid compenents of all regions

	C20:0	C20:1	C18:3n3	C21:0	C20:2	C22:0	C20:3n6	C20:4n6	C24:0
UK47	0.48	0.65	0.25	nd	nd	0.12	nd	0.02	nd
KS48	0.42	0.79	0.31	nd	nd	0.11	nd	0.02	0.07
OZ49	0.43	0.75	0.31	0.02	nd	0.12	nd	0.02	0.07
UK50	0.45	0.75	0.33	0.02	nd	0.12	nd	0.02	0.07
GB51	0.46	0.77	0.33	0.02	nd	0.12	nd	0.02	0.08
UK52	0.47	0.79	0.30	0.02	nd	0.12	nd	0.03	0.07
UK53	0.53	0.84	0.32	0.02	nd	0.16	nd	0.03	0.09
UK54	0.49	0.74	0.30	nd	nd	0.13	nd	nd	0.14
RS55	0.48	0.75	0.30	nd	nd	0.13	nd	0.03	0.10
UK56	0.48	0.62	0.25	0.01	nd	0.12	nd	0.03	0.12
UK57	0.40	0.75	0.34	0.01	nd	0.12	nd	nd	nd
UK58	0.41	0.74	0.32	nd	nd	0.14	nd	nd	nd
UK60	0.44	0.89	nd	nd	nd	0.14	nd	nd	nd
UK61	0.46	0.57	0.27	0.02	nd	0.12	nd	nd	nd
EH62	0.43	0.71	0.32	nd	nd	0.12	nd	nd	nd
EH63	0.43	0.71	0.31	nd	nd	0.12	nd	nd	nd
EH64	0.44	0.73	0.32	nd	nd	0.12	nd	nd	nd
EH65	0.42	0.73	0.31	nd	nd	0.13	nd	nd	nd
EH66	0.43	0.59	0.32	nd	nd	0.12	nd	nd	nd
EH67	0.44	0.69	0.32	nd	nd	0.13	nd	nd	nd
EH68	0.44	0.63	0.34	nd	nd	0.13	nd	nd	nd

APPENDIX B

STANDARD CALIBRATION CURVES FOR PHENOLIC COMPOUNDS

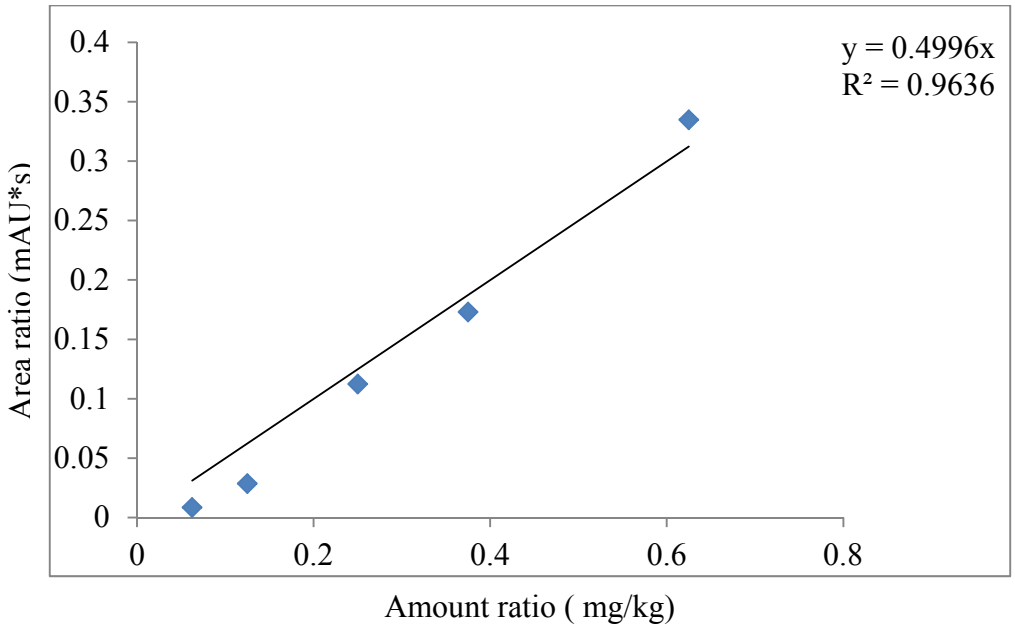

Figure A.1. Standard calibration curve for hydroxytyrosol

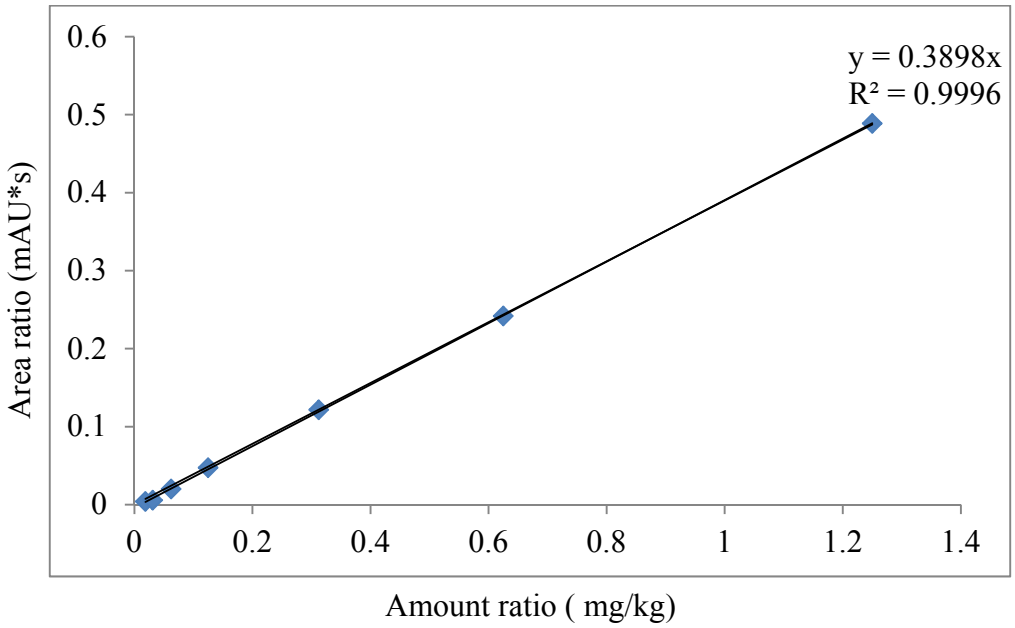

Figure A.2. Standard calibration curve for tyrosol

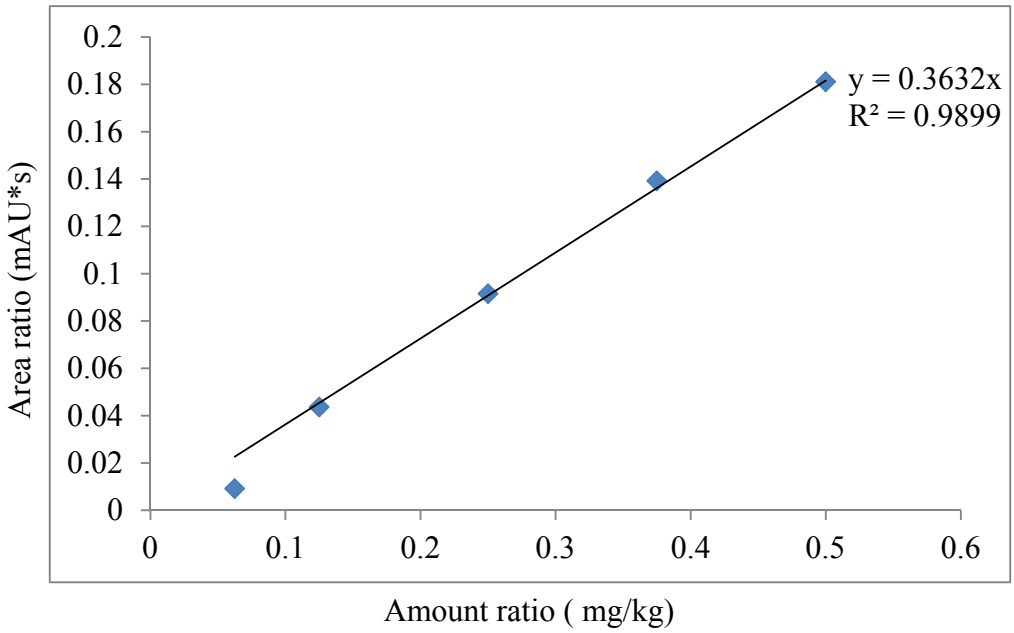

Figure A.3. Standard calibration curve for 4-hydoxyphenylacetic acid

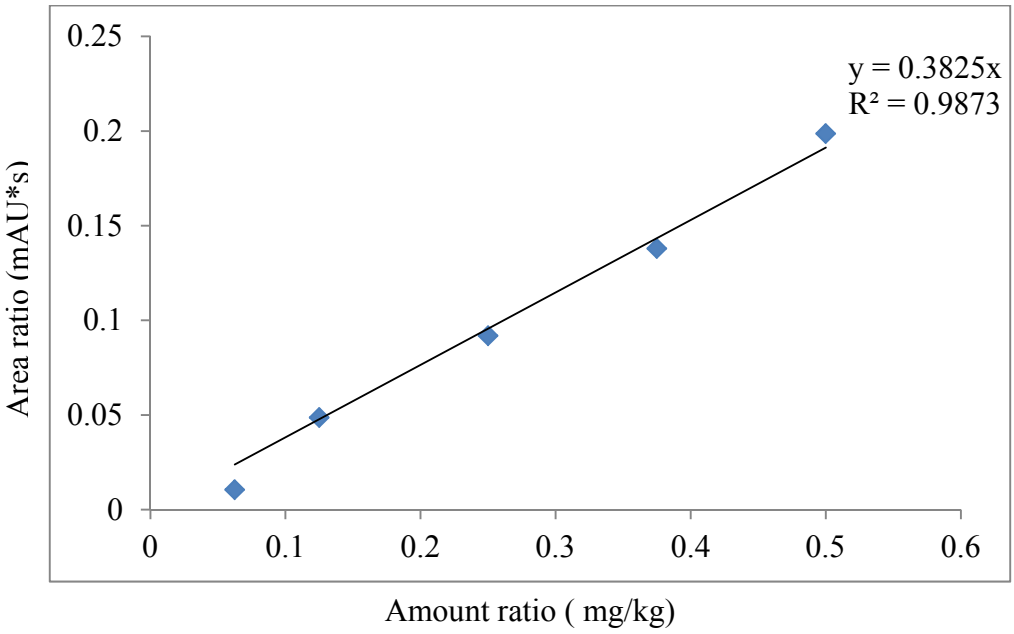

Figure A.4. Standard calibration curve for 3-hydoxyphenylacetic acid

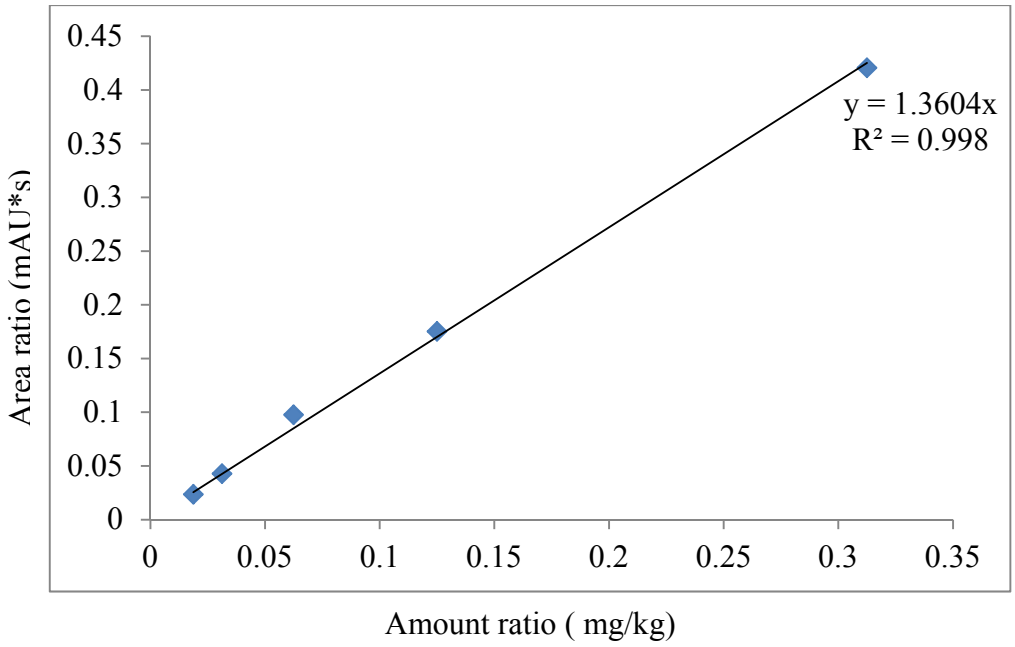

Figure A.5. Standard calibration curve for vanillic acid

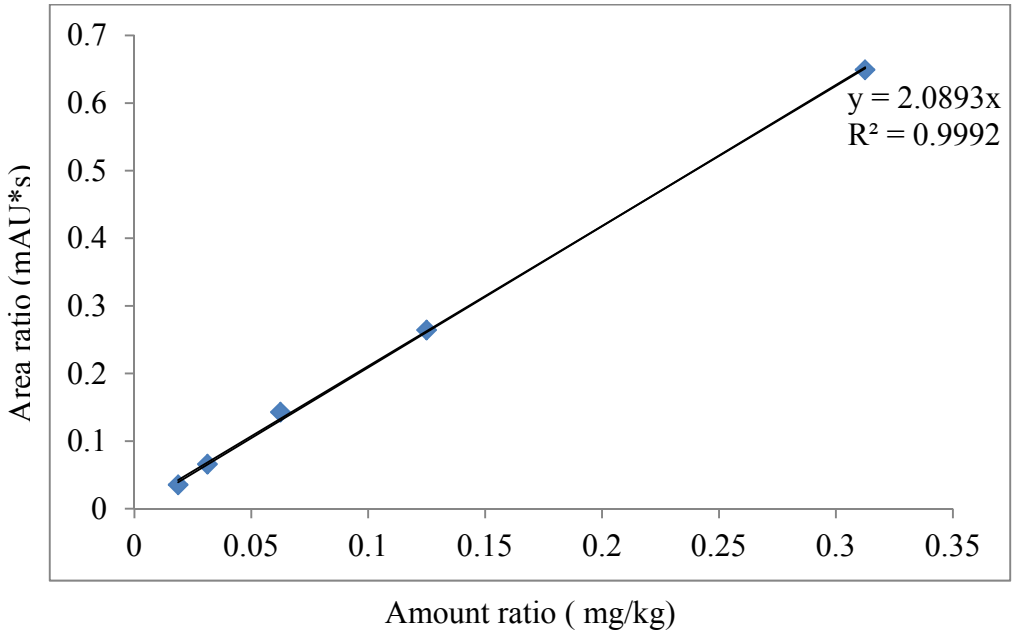

Figure A.6. Standard calibration curve for syringic acid

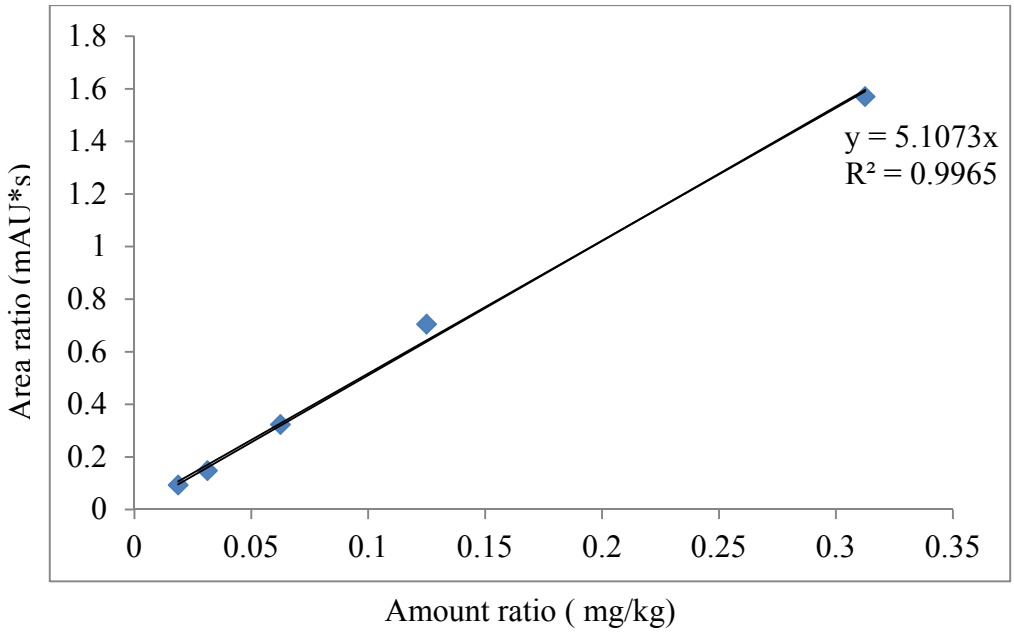

Figure A.7. Standard calibration curve for cinnamic acid

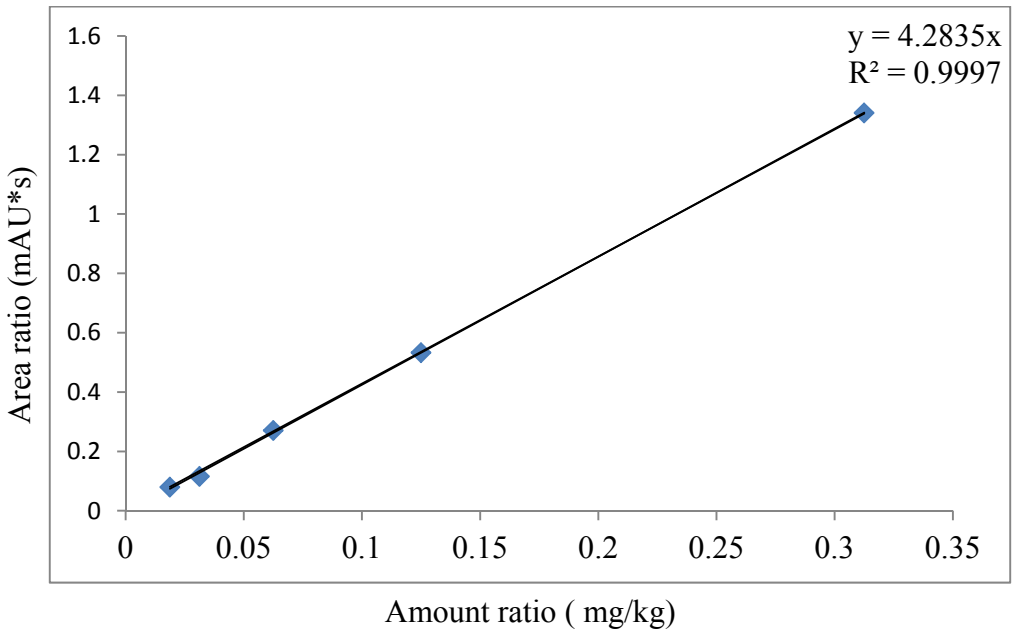

Figure A.8. Standard calibration curve for o-coumaric acid

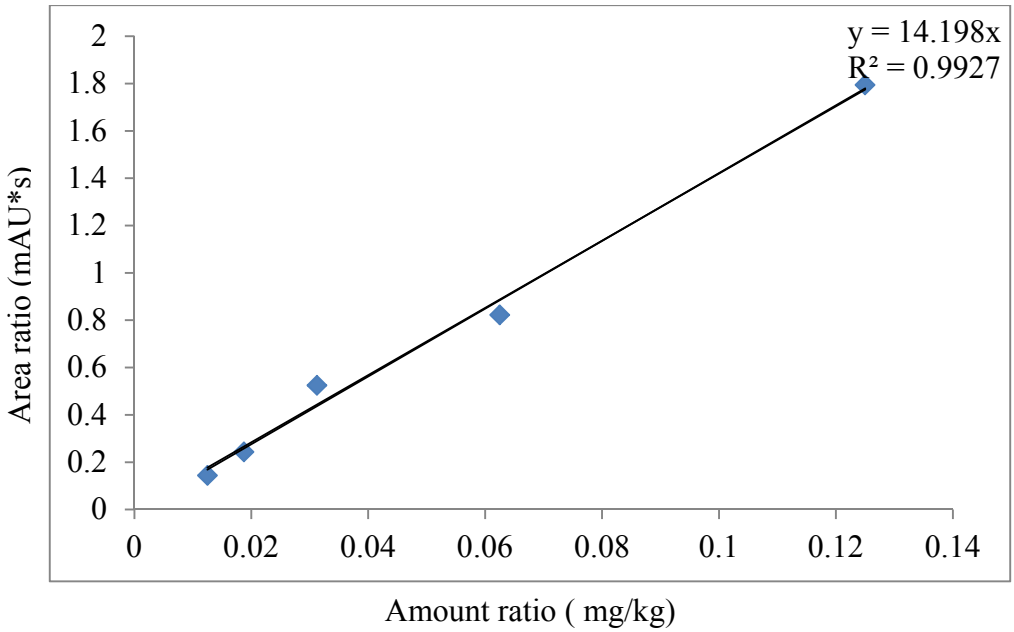

Figure A.9. Standard calibration curve for 2-3 dihdroxybenzoic acid

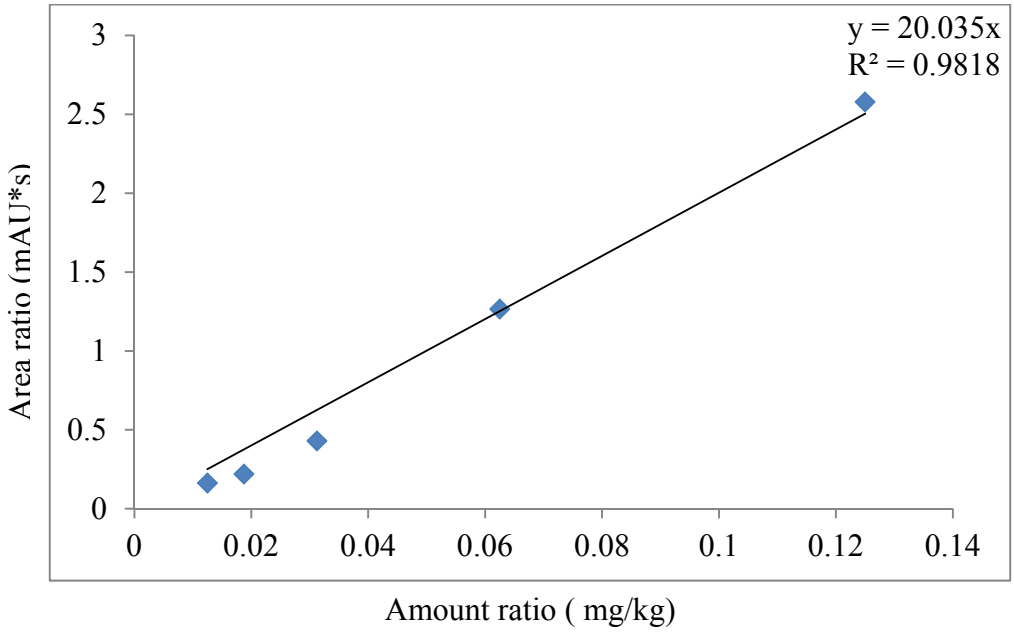

Figure A.10. Standard calibration curve for chlorogenic acid

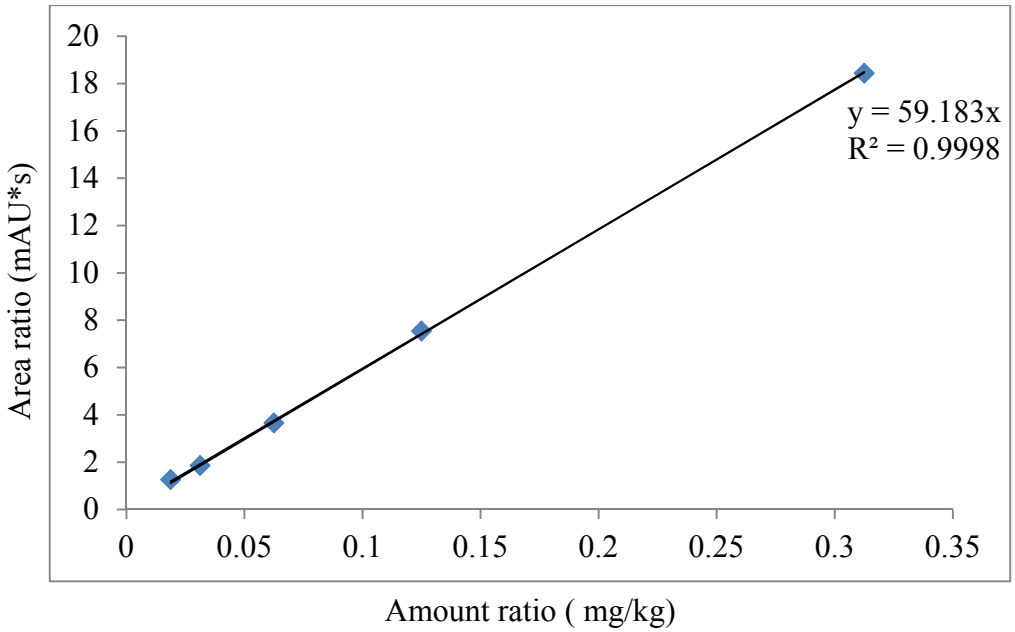

Figure A.11. Standard calibration curve for caffeic acid

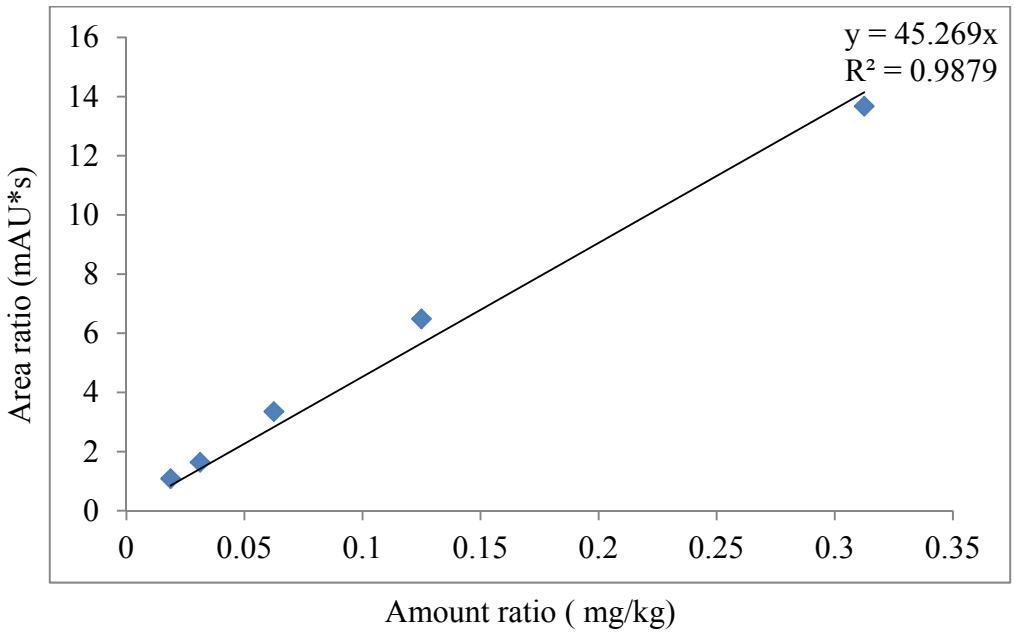

Figure A.12. Standard calibration curve for vanillin

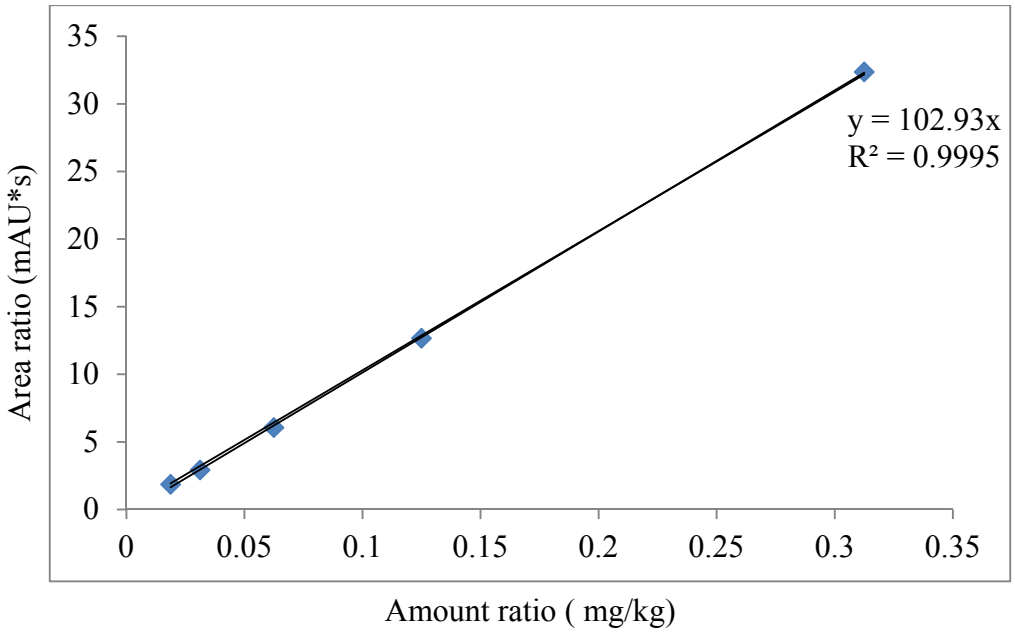

Figure A.13. Standard calibration curve for p-coumaric acid

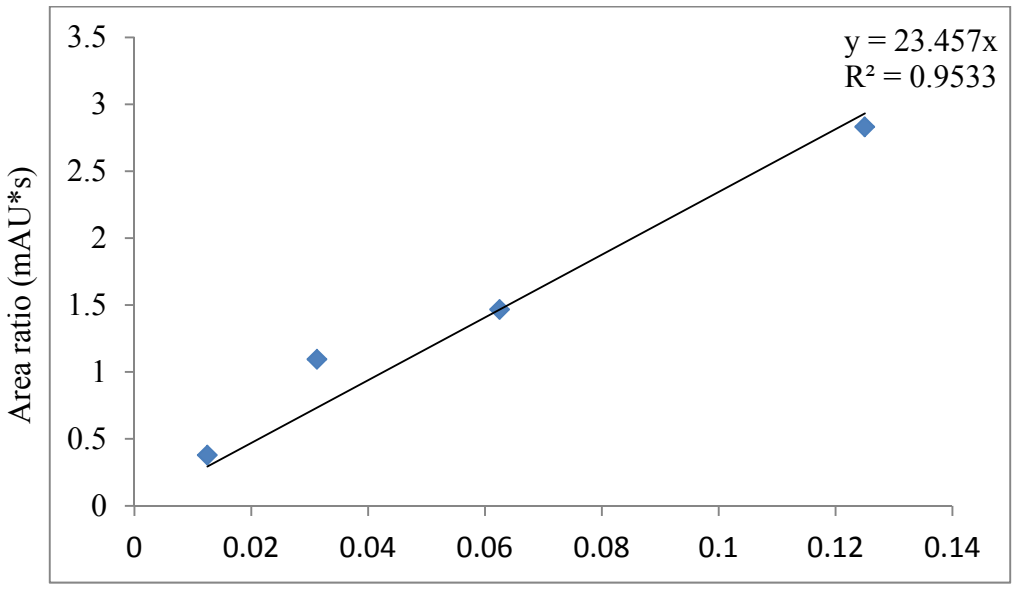

Figure A.14. Standard calibration curve for apigenin

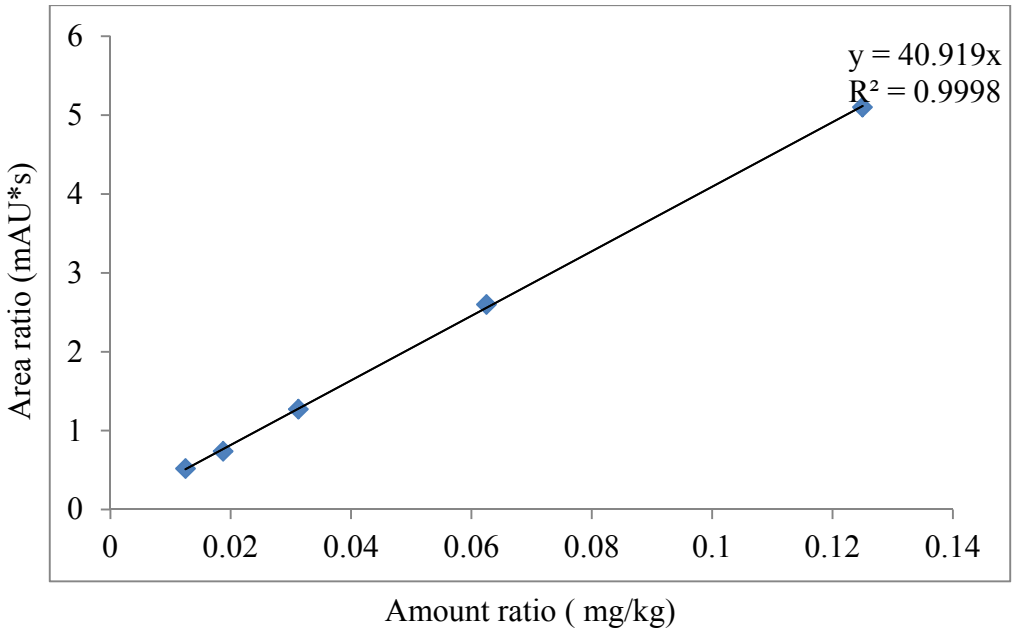

Figure A.15. Standard calibration curve for luteolin